#9
12-19-02

Source: All Sources : / . . . / : Utility Patents
Terms: "radix scutellatiae" and "fructus forsythiae" and "flos lonicerae" and antiinfluenza (Edit Search)

*Pat. No. 5834000, **

5,834,000

Nov. 10, 1998

Antiviral and antimicrobial herbal complex

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APPL-NO: 837,336

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INT-CL: [6] A01N 25#00

US-CL: 424#405; 424#195.1;

CL: 424;

SEARCH-FLD: 424#405, 195.1

REF-CITED:

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PRIM-EXMR: Levy, Neil S.

LEGAL-REP: Nixon & Vanderhye P.C.

CORE TERMS: composition, effective, ingredients, radix, tinctoria, flos, infection, patient, herbal, pharmacologically, herpes, forsythia, folium, administering, suspensa, fructus, lobata, indici, ameliorating, eliminating, japonica, mixture, illness, colds, stomach, pharmacological, undesirable, bronchitis, flu, ear

ABST:
A pharmacologically effective composition of herbs is provided which is antiviral, antibacterial, and symptom relieving for colds, flu, sinus infections, stomach infections, blocked ears due to infection, bronchitis, genital herpes, and herpes simplex. The composition does not contain any undesirable stimulants or other ingredients, such as caffeine and chlorohydrate. The preferred composition includes Isatis leaf and root, as well as other anti-microbial herbal agents, along with herbs for aches, pains, sore throat, and to reduce fever.

NO-OF-CLAIMS: 16

EXMPL-CLAIM: 1

NO-OF-FIGURES: 0

NO-DRWNG-PP: 0

SUM:

BACKGROUND AND SUMMARY OF THE INVENTION

Colds and influenza are major causes of illness and loss of productivity throughout the United States and the rest of the world. For example the National Center for Health Statistics estimates that in 1992 62 million cases of the common cold in the United States required medical attention, and that colds caused 157 million days of restricted activity and 15 million days lost from work. Approximately 10-15% of adult colds are caused by viruses also responsible for other serious illnesses, including influenza A & B viruses. It has long been considered desirable to provide an effective treatment for a wide variety of illnesses caused by viruses and bacterium, that are potent antiviral and antimicrobial agents, but also can relieve symptoms. Some traditional Chinese medicine herbal formulas can be at least somewhat effective in this regard, but they typically contain caffeine, chlorohydrate, or other components undesirable to a large segment of the population. It is desirable to provide effective herbal compositions without caffeine or other stimulants, and without chlorohydrate or like compositions.

According to the present invention a pharmacologically effective composition which is a broad spectrum antiviral and antimicrobial is provided which is completely herbal in nature and does not contain caffeine, chlorohydrate, or like undesirable components. Isatis leaf and root are the major ingredients, comprising collectively a majority of the composition. The composition may be used in the treatment of colds, infections, mumps, hepatovirus, chronic fatigue, influenza, sinus infections, stomach infections, blocked ears due to infection, bronchitis, genital herpes, and herpes simplex. The compositions according to the present invention are antiviral, antibacterial, and symptom relieving.

According to one aspect of the present invention a pharmacologically effective composition is provided comprising as the active ingredients: a mixture of Isatides tinctoria radix, Isatides tinctoria folium, Pueraria lobata radix, Forsythia suspensa fructus, Lonicera japonica flos and Chrysanthemem indici flos, in a pharmacologically effective amount. The components of the mixture preferably have the following contributions expressed in approximate weight percent:

Isatides tinctoria radix 32.5-42.5% (e.g. about 37.5%)

Isatides tinctoria folium 32.5-42.5% (e.g. about 37.5%)

Pueraria lobata radix 8-12% (e.g. about 10%)

Forsythia suspensa fructus 3-7% (e.g. about 5%)

Lonicera japonica flos 3-7% (e.g. about 5%)

Chrysanthemem indici flos 3-7% (e.g. about 5%).

Preferably the composition active ingredients consist essentially of the herbs set forth above.

The invention also relates to a method of substantially eliminating or ameliorating viral and microbial illness of human patients comprising the step of administering to a human patient in need of treatment an effective amount of a pharmacological composition such as set forth above. The invention also relates to a method of substantially eliminating or ameliorating in a human patient colds, flu, sinus infections, stomach infections, blocked ears due to infection, bronchitis, genital herpes, and herpes simplex, comprising the step of administering to a human patient in need of treatment an effective amount of a pharmacological composition as set forth above.

The invention also relates to a method of substantially eliminating or ameliorating viral and microbial illness of human patients comprising the step of administering to a human patient a pharmacologically effective composition comprising active ingredients consisting essentially of Isatis leaf and root making up the majority of the active ingredients, along with other anti-microbial herbal agents, and herbal agents for relief of aches and pains, sore throat, and to reduce fever.

DETDESC:

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The identification of specific herbal complexes that will perform effectively pharmacologically without severe side effects is a painstaking and time consuming endeavor both in the initial development of the formulation, and in its subsequent testing. Traditional Chinese medicine includes a number of formulas that can be effective for the treatment of certain illnesses, however those formulas do not necessarily transfer over to other populations with the same effectiveness, and oftentimes can contain undesirable components (such as caffeine and chlorohydrate) for a large portion of the population.

According to the present invention a broad spectrum antiviral and antimicrobial herbal composition is provided which has been found to be effective in the treatment of Western population and it does not contain undesirable ingredients such as caffeine and chlorohydrate. The compositions according to the present invention use Isatis leaf and root as the major ingredients. These herbs have been used in traditional Chinese medicine for colds, virus, infection, cancer, mumps, hepatovirus, and chronic fatigue. These components are utilized in the composition according to the present invention along with other potent herbal antimicrobial agents, and along with herbs for relief of aches and pains, and sore throat, and to reduce fevers. The herbal compositions according to the present invention are antiviral, antibacterial, and symptom relieving.

According to the present invention a pharmacologically effective composition is provided comprising as active ingredients a mixture of Isatides tinctoria radix, Isatides tinctoria folium, Pueraria lobata radix, Forsythia suspensa fructus, Lonicera japonica flos and Chrysanthemem indici flos, in a pharmacologically effective amount. The components of the mixture preferably have the following contributions expressed in approximate weight percent:

Isatides tinctoria radix	32.5-42.5% (e.g. about 37.5%)
Isatides tinctoria folium	32.5-42.5% (e.g. about 37.5%)
Pueraria lobata radix	8-12% (e.g. about 10%)
Forsythia suspensa fructus	3-7% (e.g. about 5%)
Lonicera japonica flos	3-7% (e.g. about 5%)
Chrysanthemem indici flos	3-7% (e.g. about 5%).

"Isatides tinctoria radix" is also known as indigo, or cruciferae, or Radix Isatidis, whereas "Isatides tinctoria folium" is also known as indigo, cruciferae, and folium Isatides. Active ingredients are believed to be indirubin, sinigrin, indical, indoxyl, isatan, and labenzyme.

"Pueraria lobata radix" is also known as Pueraria, leguminosae, radix puerariae, and by the common name "kudzu". Active ingredients are believed to be daidzin, daizein, and puerarin. In the composition according to the invention it relieves fever, congestion, and other symptoms, without undesirable side effects.

"Forsythia suspensa fructus" comprises halfshells of the fruit of forsythia, also known as fructose forsythiae, and Oleaceae. Active constituents are believed to be phillyrin and other

saponin and flavonol glycosides. In the composition of the invention it relieves the symptoms of sore throat and reduces fever without undesirable side effects.

"Lonicera japonica flos" comprises the whole flower buds of Lonicera, also known as Flos Lonicerae, and Caprifolaceae. Active ingredients are believed to be luteolin glucoside, lonicerin, and inositol. This herb is a potent anti-microbial and also relieves the symptoms of sore throat, and can reduce fever.

"Chrysanthemem indici flos" is also known as the whole flowers of chrysanthemum, or Flos Chrysanthemi, or Compositae. Active constituents are believed to be apigenin, borneol, and a variety of paraffins. In the compositions according to the invention this herbal agent soothes a number of aches, pains, and other symptoms of colds, flu, and the like, without side effects.

In addition to the active ingredients, the herbal complexes utilized according to the present invention may have any number of substantially inert ingredients which will vary depending upon the particular form by which the complex will be administered. Normally the complex is administered in the form of ingestible tablets or capsules which are swallowed with water, although the complex active ingredients may be mixed with food or beverage items and eaten or drunk, or in extreme cases may be introduced directly into the bloodstream using a hypodermic needle, I.V., or the like. The dose may vary depending upon the size, age, and condition of the patient being treated and the particular percentages of components herbal complex utilized, but normally between about 500-3000 mg of active herbal complex is administered per day, with part of the total dose preferably taken at two or more different times during the day.

A typical manner of processing herbs to produce the complex may be as follows, although a wide variety of different known processing techniques may be utilized depending upon the exact form of the material desired, and the availability of material or equipment:

The powder end product of the complex is typically a 1:1 extract. Testing of raw materials used is conducted using standard organoleptic, High performance Liquid Chromatography, and microbiologic methods. The solvent mixture used for extractions for herbs used in the complex is about 95% SDA-3C and about 5% potable water. SDA-3C is specifically denatured alcohol composed of 95% ethanol and 5% isopropyl. The extraction method is thermokinetic maceration, specifically about 180o F. for about three hours, plus warm up and cool down.

Following extraction, a sample is tested for the percentage of dissolved solids recovered. This is compared with the specified standards and, when necessary, the processing is continued until the standards are reached. The base material of the extract is marc; no rinse of the extracted powder is required. The miscella is distilled. The distilled total miscella is dehydrated onto the base material. This receives a final milling (1/32" screen) in a sanitary stainless mill, using a vacuum system to transport the product directly into the final containers. Samples are taken for quality control tests which are visual, taste, microbiologic and High Performance Liquid chromatography. Samples are also taken for permanent record. That material is readily made into tablets, or placed in ingestible capsules, e.g. about 300 mg per capsule.

Another possible technique is as follows:

The powder and end product of this formula is typically a 1:1 extract. Testing of raw materials used is conducted using standard organoleptic, High Performance Liquid Chromatography and microbiologic methods. The solvent solution is preferably about 95% SDA-3C and 5% water.

The herb and the solvent are added together in the extract processor for processing. The supernatant liquid of solvent and solids is drained into the holding/settling tank where the volume is measured and the solids content is determined by analysis. Samples are drawn of both and liquid supernatant and sediment for microbiologic testing. The supernatant liquid is pumped through a 100 mesh liquid filter into the Sanitizing vessel. The liquid is processed

for a minimum of four hours at the boiling temperature of about 178o F. The volume of the liquid is measured and a solids analysis is done. A sample is drawn for microbiologic testing. The liquid is pumped through a 100 mesh filter and sprayed into the vacuum dryer, using volume and solids data to adjust the product to the desired concentration for the finished product. The resulting material is dried. The processor is emptied into sanitary bulk bins or barrels and transported to milling. A pre-grind sample is drawn for biologic testing. The material is milled in a sanitary stainless steel milling system using a 1/16" screen. The material is unloaded from the mill system directly via Vac-u-Max collector into double lined 44 gallon fiber drums. A sample is drawn from each container for biologic testing. Typical microbiologic requirements are:

*	Limits
Aerobes	max. 10,000/g
Coliform	negative
Salmonella	negative
E. Coli	negative
Yeast	max. 100/g
Mold	max. 100/g

The utilization of complex herbal formulations as set forth above for the elimination or amelioration of a number of viral or bacteria related illnesses, such as colds, flu, sinus infections, stomach infections, blocked ears due to infections, bronchitis, genital herpes, and herpes simplex, has been shown to be effective through testing. A pharmacologically effective composition comprising as active ingredients a mixture having the following contributions expressed in weight percent:

Isatides tinctoria radix	about 37.5%
Isatides tinctoria folium	about 37.5%
Pueraria lobata radix	about 10%
Forsythia suspensa fructus	about 5%
Lonicera japonica flos	about 5%
Chrysanthemem indici flos	about 5%,

has been tested as indicated by the following table (each capsule contained about 300 mg active ingredients):

Clinical Data Table

Log #	Patient Age & Gender	* Dosage Time	Treatment Duration	Presenting Symptoms	* Resultant Effects	Side Effects
304	54 yrs female	1 capsule 3 x daily *	10 days *	stomach "flu" *	stomach much improved	none
305	late 50s male *	1 capsule 3 x daily *	3 weeks *	bacterial infection in his stomach	"it is the only medication that worked on his	none

	*	*	*	*	stomach	
	*	*	*	*	problem"	
306	mid 30s	3 capsules 3 x	5 days	residual ear	his ears	none
	male	daily	*	blockage after	completely	
	*	*	*	the flu	cleared up	
308	49 years	2 capsules 3 x	1 week	chronic	bronchitis cleared	none
	male	daily	*	bronchitis		
	*	*	*	since 1973	up	

In the above table, each capsule contained approximately 300 mg of the herbal complex. The herbal formulations according to the invention are effective when administered alone, but can be administered with other pharmacological treatments typically without adverse side effects.

It is highly desirable that the herbal compositions according to the present invention consist essentially of herbal agents, and do not have undesirable materials such as those that are primarily stimulants, depressants, or the like, as active ingredients (and that any "inert" ingredients added also do not include undesirable materials such as depressants or stimulants). Particularly to be avoided are stimulants such as caffeine, and chlorohydrate or like materials.

Thus it will be seen that according to the present invention broad spectrum antiviral and antimicrobial herbal complexes are provided which are effective but which have few or no side effects, and do not require stimulants or other undesirable ingredients to be effective. While the invention has been herein shown and described in what is presently conceived to be the most practical and preferred embodiment thereof it will be apparent to those of ordinary skill in the art that many modifications may be made thereof within the scope of the invention, which scope is to be accorded the broadest interpretation of the appended claims so as to encompass all equivalent methods and products.

CLAIMS: What is claimed is:

[*1] 1. A pharmacologically effective composition comprising as active ingredients a mixture of Isatides tinctoria radix, Isatides tinctoria folium, Pueraria lobata radix, Forsythia suspensa fructus, Lonicera japonica flos and Chrysanthemem indici flos, in a pharmacologically effective amount, wherein the components of the mixture have the following contributions expressed in approximate weight percent:

Isatides tinctoria radix	32.5-42.5%
Isatides tinctoria folium	32.5-42.5%
Pueraria lobata radix	8-12%
Forsythia suspensa fructus	3-7%
Lonicera japonica flos	3-7%
Chrysanthemem indici flos	3-7%.

[*2] 2. A composition as recited in claim 1 wherein said active ingredients consist essentially of Isatides tinctoria radix, Isatides tinctoria folium, Pueraria lobata radix, Forsythia suspensa fructus, Lonicera japonica flos and Chrysanthemem indici flos.

[*3] 3. A composition as recited in claim 2 wherein the components of the mixture have the following contributions expressed in approximate weight percent:

Isatides tinctoria radix	32.5-42.5%
Isatides tinctoria folium	32.5-42.5%
Pueraria lobata radix	8-12%
Forsythia suspensa fructus	3-7%
Lonicera japonica flos	3-7%
Chrysanthemem indici flos	3-7%.

[*4] 4. A method of substantially eliminating or ameliorating viral and microbial illness of human patients comprising the step of administering to a human patient in need of treatment an effective amount of the pharmacological composition of claim 1.

[*5] 5. A method of substantially eliminating or ameliorating viral and microbial illness of human patients comprising the step of administering to a human patient in need of treatment an effective amount of the pharmacological composition comprising as active ingredients a mixture of Isatides tinctoria radix, Isatides tinctoria folium, Pueraria lobata radix, Forsythia suspensa fructus, Lonicera japonica flos and Chrysanthemem indici flos, in a pharmacologically effective amount.

[*6] 6. A method of substantially eliminating or ameliorating viral and microbial illness of human patients comprising the step of administering to a human patient in need of treatment an effective amount of the pharmacological composition of claim 2.

[*7] 7. A method of substantially eliminating or ameliorating viral and microbial illness of human patients comprising the step of administering to a human patient in need of treatment an effective amount of the pharmacological composition of claim 3.

[*8] 8. A method of substantially eliminating or ameliorating in a human patient colds, flu, sinus infections, stomach infections, blocked ears due to infection, bronchitis, genital herpes, and herpes simplex, comprising the step of administering to a human patient in need of treatment an effective amount of the pharmacological composition of claim 1.

[*9] 9. A method of substantially eliminating or ameliorating in a human patient colds, flu, sinus infections, stomach infections, blocked ears due to infection, bronchitis, genital herpes, and herpes simplex, comprising the step of administering to a human patient in need of treatment an effective amount of the pharmacological composition comprising as active ingredients a mixture of Isatides tinctoria radix, Isatides tinctoria folium, Pueraria lobata radix, Forsythia suspensa fructus, Lonicera janonica flos and Chrysanthemem indici flos, in a pharmacologically effective amount.

[*10] 10. A method of substantially eliminating or ameliorating in a human patient colds, flu, sinus infections, stomach infections, blocked ears due to infection, bronchitis, genital herpes, and herpes simplex, comprising the step of administering to a human patient in need of treatment an effective amount of the pharmacological composition of claim 2.

[*11] 11. A method of substantially eliminating or ameliorating in a human patient colds, flu, sinus infections, stomach infections, blocked ears due to infection, bronchitis, genital herpes, and herpes simplex, comprising the step of administering to a human patient in need of treatment an effective amount of the pharmacological composition of claim 3.

[*12] 12. A composition as recited in claim 1 wherein the components of the mixture have the following contributions expressed in weight percent:

Isatides tinctoria radix	about 37.5%
Isatides tinctoria folium	about 37.5%
Pueraria lobata radix	about 10%
Forsythia suspensa fructus	about 5%
Lonicera japonica flos	about 5%
Chrysanthemem indici flos	about 5%.

[*13] 13. A composition as recited in claim 2 wherein the components of the mixture have the following contributions expressed in weight percent:

Isatides tinctoria radix	about 37.5%
Isatides tinctoria folium	about 37.5%
Pueraria lobata radix	about 10%
Forsythia suspensa fructus	about 5%
Lonicera japonica flos	about 5%
Chrysanthemem indici flos	about 5%.

[*14] 14. A method of substantially eliminating or ameliorating viral and microbial illness of human patients comprising the step of administering to a human patient a pharmacologically effective composition having active ingredients consisting essentially of Isatides leaf and root making up the majority of the active ingredients, along with other anti-microbial herbal agents, and herbal agents for relief of aches and pains, sore throat, and to reduce fever.

[*15] 15. A method as recited in claim 14 wherein said method is practiced by administering a pharmacologically effective composition in which the Isatides leaf and root collectively comprise between 65-85% of the active ingredients.

[*16] 16. A method of substantially eliminating or ameliorating viral and microbial illness of human patients comprising the step of administering to a human patient a pharmacologically effective composition comprising active ingredients comprising Isatides leaf and root making up the majority of the active ingredients, along with other anti-microbial herbal agents, and herbal agents for relief of aches and pains, sore throat, and to reduce fever; and wherein said composition is devoid of caffeine or like stimulants, and devoid of chlorohydrate or like compositions.

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, LEVEL 1 - 1 OF 1 PATENT

5,443,839

<=2> GET 1st DRAWING SHEET OF 2

Aug. 22, 1995

Liposomes containing scutellaria extracts

<PIRATION-DATE: Aug. 22, 1999 due to failure to pay maintenance fees

NVENTOR: Meybeck, Alain, Courbevoie, France

SSIGNEE-AT-ISSUE: LVMH Recherche, Colombes Cedex, France (03)

PPL-NO: 835,004

LED: Feb. 12, 1992

OR-PRIOR:
Mar. 9, 1988 France 88 03066

EL-US-DATA:
Continuation of Ser. No. 576,441, Sep. 7, 1990 now abandoned

T-CL: [6] A61K 9#127

-CL: 424#450; 424#401; 424#195.1

: 424

ARCH-FLD: 424#450, 195.1, 401

EF-CITED:

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RIM-EXMR: Kishore, Gollamudi S.

EGAL-REP: Cave; Bryan

ORD TERMS: extract, liposome, composition, ogon, scutellaria, phase, lamellar, el, hydrated, batch, root, lipid, lipidic, solvent, extraction, cosmetic, nti-allergic, organic, dry, baicaline, baicaleine, oil-in-water, suspension, ixture, croton, oil, anti-inflammatory, pharmaceutical, gelled, 100

ABST:
A composition having anti-inflammatory, anti-allergic or anti-aging activity omprising hydrated lipidic lamellar phases or liposomes containing an extract f Scutellaria and a method for treating inflammation, allergies or aging by opical administration of the composition.

)-OF-CLAIMS: 18

L-CLAIM: <=3> 1

)-OF-FIGURES: 2

)-DRWNG-PP: 2

RCASE:

PARCASE:

This is a continuation of U.S. Application Ser. No. 07/576,441, filed as PCT/FR89/00020, Jan. 23, 1989, now abandoned, which is incorporated by reference herein.

SUM:

The present invention essentially relates to a composition based on hydrated l.. .d lamellar phases or liposomes containing an extract of Scutellaria, or at least one of its constituents, and a cosmetic or pharmaceutical, particularly dermatological composition, with anti-allergic, anti-inflammatory, anti-ageing activity, incorporating it.

The plant varieties of Scutellaria of the Labiatae family include without limitation Scutellaria Baicalensis, Scutellaria Viscidula or Scutellaria Galericulata.

The most well known extracts of Scutellaria are extracts of roots of Scutellaria Baicalensis Georgi (Scutellaria Radix) also named "OGON" or even "OUGON".

Extracts of Ogon as defined hereinabove are widely described in the prior art for cosmetic, pharmaceutical and horticultural use.

Japanese Patent Application JP-57-209895 published under No. JP-A-59-101412 describes a cosmetic composition for hair protection containing an extract of Ogon (Scutellaria Baicalensis root).

Similarly, Japanese Patent Application JP-57-183419 published under No. JP-A-59-73509 describes a cosmetic composition containing Ogon in powder form at a rate of 0.005-2% by weight, or an extract thereof as an essential component, this composition presenting an excellent effect of improving dry skins, spots, wrinkles, etc. This Application specifies that the Ogon is constituted by dried roots of Scutellaria Baicalensis Georgi or of related plants.

The use of extract of Scutellaria Radix for preparing a cosmetic composition avoiding the formation of spots on the skin is also described in Japanese Patent A.. ication JP-59-241641 published under No. JP-A-61-122209, in mixture with .. .r active components.

The use of Scutellaria as one of the components of a multi-component extract for the formation of a skin reactivator is described in Japanese Patent Application JP-54-172382 published under No. JP-A-56-92821.

Japanese Patent Application JP-54-34771 published under No. JP-A-55-127309 lso describes a cosmetic composition comprising an extract of Scutellaria aicalensis Georgi for the prevention of hardness, sun burns and inflammation of he skin. The cosmetic formulation may be used in the form of lotion, cream, mulsion, cleaning cream and soap.

Pharmaceutical compositions containing Ogon are described respectively in: -62-26229 (Scutellaria Radix, agent for promoting differentiation of the ous cells); JP-A-61-167623 (Ougon, root of Scutellaria Baicalensis, agent or inhibiting coagulation of the platelets); JP-A-61-161219 (Scutellaria aicalensis Georgi or Scutellaria Viscidula Buxge, treatment of atopical ermatites); JP-A-61-109733 (Ougon, root of Scutellaria Baicalensis, arcinostatic agent); JP-A-61-263923 (2-interleucine inductor of low toxicity ontaining, in a mixture of herb extract, an extract of Ogon); JP-A-58-121218 composition for the control of tooth decay comprising, inter alia, an extract f Scutellaria Baicalensis; GB-A-1096708 (anti-narcotic drug containing, inter lia, roots of Scutellaria Baicalensis Georgi (5%)); JP-A-62-033125 (drug mproving the anti-cancerous effect of Tegafur containing, inter alia, roots of cutellaria in powder form).

Extracts of Ogon have also been used in agriculture for the production of an gent activating plant growth (JP-A-61-115009); as fungicide (JP-A-56-022709); r as plant disease control agent (JP-A-62-129209).

Likewise, certain active agents have been extracted from Ogon, such as aicaline as constituent for the treatment of allergic diseases (cf. P-A-61-50921), baicaline or baicaleine also as anti-allergic agent JP-A-61-50918), as well as Planta Medica, J. Medici. Plant Research 1981, vol. 3, pages 194-201. As deodorant component, the baicaleine or baicaline has also een used (JP-A-61-268259); derivatives of baicaleine in the form of salt and of emi-ester as anti-inflammatory or anti-asthmatic agent are described in P-A-70-25716 = U.S. Pat. No. 3,549,662. The use of wogonine and of baicaline s described in JP-A-48-68717 for the treatment of arteriosclerosis, apoplexy nd hypercholesterolaemia.

The use of baicaline, of wogonine, etc., is described for the preparation of nthocyanic pigment in JP-A-55-13711.

inally, the use of Scutellaria in cosmetic compositions for retaining water n the skin is described in JP-A-60-258104.

It may thus be ascertained that the use of Scutellaria and in particular of gon extract, or of various constituents thereof, is widely described in the osmetic or pharmaceutical fields.

cosmetic or pharmaceutical fields.

Furthermore, the use of hydrated lipid lamellar phases or liposomes in pharmaceutical compositions or cosmetic compositions, in which various active ingredients are incorporated, is already known (FR-A-2 540 381).

It has now been quite surprizingly and unexpectedly discovered that the incorporation of the above-mentioned extract of Scutellaria, in particular of Ogon extract, or of active substance having been able to be isolated from such an extract of Ogon obtained by chemical synthesis, selected in particular from: ',5,7-trihydroxy-8-methoxy-flavone (or 2'-hydroxy-wogonine), ',5-dihydroxy-6,6',7,8-tetramethoxy-flavone (or skullcap flavone II or eobaicaleine), 2',5,5',7-tetrahydroxy-6',8-dimethoxy-flavone, -hydroxy-8-methoxy-flavone-7-O-D-glucuronide (or wogonin-7-O-D-glucuronide or roxindine), 5-hydroxy-7,8-dimethoxy-flavone (or 7-O-methyl-wogonine), ,7-dihydroxy-6-methoxy-flavone (or oroxyline A or 6-O-methyl-baicaleine), ',5,7-trihydroxy-8-methoxy-flavone (or 4'-hydroxy-wogonine), ',5,6'-trihydroxy-7,8-dimethoxy-flavone, 5,7,8-trihydroxy-flavone (or orwogonine), 5,6,7-trihydroxy-flavone (or baicaleine), ,8-dihydroxy-6,7-dimethoxy-flavone, 2',3,5,6',7-pentahydroxy-flavone, ',5,6-trihydroxy-flavone-7-O-D-glucuronide (or 4'-hydroxy-baicaline, ,6-dihydroxy-flavone-7-O-D-glucuronic methyl ester acid (or baicaline methyl ster), 2',5,7-trihydroxy-flavone (2'-hydroxy chrysine), ,7-dihydroxy-8-methoxy-flavone (or wogonine), ',5,7-trihydroxy-6',8-dimethoxy-flavone (or 2'-hydroxy-6'-methoxy-wogonine), ',5,6,7-tetrahydroxy-flavone (or 4'-hydroxy-baicaleine), ,6-dihydroxy-flavone-7-O-D-glucoside (baicaleine-7-O-D-glucoside), -hydroxy-4',6,7-trimethoxy-flavone (or salvigenine), -hydroxy-6-methoxy-flavone-7-O-D-glucuronide (or oroxyline -7-O-D-glucuronide), 5,6-dihydroxy-flavone-7-O-D-glucuronide (or baicaline), -hydroxy-6-methoxy-flavone-7-O-D-glucuronic methyl ester acid (or oroxindine ethyl ester), 5,7-dihydroxy-flavone (or chrysine), and preferably from: ogonine, 2'-hydroxy-wogonine, baicaleine, neobaicaleine, oroxindine, baicaline, t least partly in a hydrated lipid lamellar phase or in liposomes, provoked a ore intensive activity of this extract of these substances. This concerns all he activities known for the extracts of Ogon or the substances isolated from uch extracts such as the substances mentioned above. An even more radical ovement in activity has been observed in the anti-inflammatory, ti-allergic activity and anti-ageing activity.

An effect of synergy may thus to some extent be deduced therefrom for ncorporations of extract of Scutellaria, in particular of Ogon extract or ctive substances isolated from such extracts, such as the substances entioned above, in hydrated lipid lamellar phases or in liposomes.

mentioned above, in hydrated lipid lamellar phases or in liposomes.

The present invention thus has for its object to solve the new technical problem consisting in supplying a novel formulation of extract of Scutellaria, in particular of Ogon extract, or of any active substance isolated from such an extract or reconstituted by chemical synthesis, making it possible to potentialize their effectiveness in order to allow use thereof in cosmetic or pharmaceutical, particularly dermatological compositions, with anti-inflammatory, anti-allergic or anti-ageing activity.

The present invention solves this new technical problem for the first time, in satisfactory manner.

Thus, according to a first aspect, the present invention furnishes a composition based on hydrated lipid lamellar phases or liposomes, characterized in that said hydrated lipid lamellar phases or said liposomes contain at least in part an extract of Scutellaria, or at least an active substance isolated from such an extract or obtained by chemical synthesis, in particular from: 2',5,7-trihydroxy-8-methoxy-flavone (or 2'-hydroxy-wogonine), 2',5-dihydroxy-6,6',7,8-tetramethoxy-flavone (or skullcap flavone II or neobaicaleine), 2',5,5',7-tetrahydroxy-6',8-dimethoxy-flavone, 5-hydroxy-8-methoxy-flavone-7-O-D-glucuronide (or wogonin-7-O-D-glucuronide or oroxindine), 5-hydroxy-7,8-dimethoxy-flavone (or 7-O-methyl-wogonine), 5,7-dihydroxy-6-methoxy-flavone (or oroxyline A or 6-O-methyl-baicaleine), 4',5,7-trihydroxy-8-methoxy-flavone (or 4'-hydroxy-wogonine), 2',5,6'-trihydroxy-7,8-dimethoxy-flavone, 5,7,8-trihydroxy-flavone (or norwogonine), 5,6,7-trihydroxy-flavone (or baicaleine), 5,8-dihydroxy-6,7-dimethoxy-flavone, 2',3,5,6',7-pentahydroxy-flavone, 4',5,6-trihydroxy-flavone-7-O-D-glucuronide (or 4'-hydroxy-baicaline), 5,6-dihydroxy-flavone-7-O-D-glucuronic methyl ester acid (or baicaline methyl ester), 2',5,7-trihydroxy-flavone (2'-hydroxy chrysine), 5,7-dihydroxy-8-methoxy-flavone (or wogonine), 2',5,7-trihydroxy-6',8-dimethoxy-flavone (or 2'-hydroxy-6'-methoxy-wogonine), 4',5,6,7-tetrahydroxy-flavone (or 4'-hydroxy-baicaleine), 5,6-dihydroxy-flavone-7-O-D-glucoside (baicaleine-7-O-D-glucoside), 5-hydroxy-4',6,7-trimethoxy-flavone (or salvigenine), 5-hydroxy-6-methoxy-flavone-7-O-D-glucuronide (or oroxyline A-7-O-D-glucuronide), 5,6-dihydroxy-flavone-7-O-D-glucuronide (or baicaline), 5,7-dihydroxy-6-methoxy-flavone-7-O-D-glucuronic methyl ester acid (or oroxindine methyl ester), 5,7-dihydroxy-flavone (or chrysine).

For a precise description of these isolated substances, reference may be made to the description of the prior art, particularly to Planta Medica, Journal of Medicinal Plant Research (1981), vol. 43, pages 194-201, likewise in Chem.

Medicinal Plant Research (1981), vol. 43, pages 194-201, likewise in Chem. Pharm. Bull., (1984), vol. 32, pages 5051-5054, or Chem. Pharm. Bull. (1988), vol. 36, No. 2, pages 654-661.

The chemical structures of eight flavonoid compounds isolated from Scutellaria baicalensis Georgi were presented in Planta Medica at pages 195 and 197 and are set forth below: [See Original Patent for Chemical Structure Diagram] I: Wogonin R = HVII: R = glucuronic acid [See Original Patent for Chemical Structure Diagram] II: Oroxylin A [See Original Patent for Chemical Structure Diagram] III: Skullcapflavone I [See Original Patent for Chemical Structure Diagram] IV: Skullcapflavone II [See Original Patent for Chemical Structure Diagram] V: Baicalein R = HVI: Baicalin R = glucuronic acid [See Original Patent for Chemical Structure Diagram] VIII: 2(S),2',5,6',7-tetrahydroxyflavanone

The chemical properties of wogonin (I), skullcapflavone II (IV), baicalein (V), and 2(S),2',5,6',7-tetrahydroxy-flavanone (VIII) are described on page 195 of Planta Medica as follows:

Wogonin (I)

Yellow needles from n-hexane: EtOAc, mp. 202o-203o, [Lit. mp. 203o] [1]. In the IR and PMR spectra data, it was identical with authentic sample of wogonin. Yield 25 g.

Skullcapflavone II (IV)

Yellow prisms from EtOAc. mp. 180o [Lit. mp. 180o-181o] [Mg + HCl: Orange Yellow. PMR (in DMSO-d6) delta ppm: 3.76, 3.80. 3.82, 4.00 (3H, each singlet, OCH3), 6.22 (1H, singlet, C3-H), 6.56 (2H, doublet, J = 8.0 Hz, C3-H and C3-H), 8.24 (1H, triplet, J = 8.0 Hz, C4-H), 10.0 (1H, broad singlet, C2-OH), 12.67 (1H, singlet, C3-OH). IR nu max <nujol > cm: 3200-3100 (OH), 1650 (C=O), 1600, 1560 (aromatic ring). UV lambda max <EtOH > nm (log epsilon): 270 (4.46). From the PMR spectra data it was identical with that reported for skullcapflavone II [1]. Yield 500 mg.

Baicalin (V)

Yellow prisms from CHCl3: MeOH, mp. 263o (decomp.) [Lit. mp. 265o] [2]. From the IR and PMR spectra data it was identical with an authentic sample of baicalein. Yield 53 g.

Baicalin (VI)

Baicalin (VI)

Yellow crystalline powder from MeOH, mp. 220o (decomp.) [Lit. mp. 223o] [2]. From the IR and PMR spectra data it was identical with an authentic sample of baicalin and the melting point showed no depression on admixture with an authentic sample. Baicalin, which was required for comparison of antibacterial effects, was isolated from the MeOH extract of roots of S. baicalensis.

(S),2',5,6',7-tetrahydroxyflavanone (VIII)

Colorless prisms from n-hexane: EtOAc, mp. 240o (decomp.), Mg + HCl: Orange yellow, 1% Ce (SO4)2 (10% H2SO4): Orange, FeCl3 reagent: dark green. [alpha] D <20o> + 6.13o (c = 1.012, MeOH). MS m/e 288 (M< + >). Anal. Calcd. for C13H12O6: C, 62.50; H, 4.20; mol. wt., 288.25. Found: C. 62.54; H, 4.22. IR, nu max <nujol > cm< - 1> : 3450, 3200 (OH), 1640 (chelated C=O), 1610, 1517 (aromatic ring). UV lambda max <EtOH > nm (log epsilon): 289 (4.21); UV lambda max <EtOH(AlCl> S 3<) > nm: 312; UV lambda max <EtOH(AcONa) > nm: :327.

PMR (in DMSO-d6) delta ppm: 2.35 (1H, doublet. J = 4.0 Hz. C3-H), 3.40 (1H, quartet, J = 14.0 Hz. 17.0 Hz, C3-H), 5.83, 5.87 (1H, each doublet, J = 2.5 Hz, aromatic H), 5.84 (1H, quartet, J = 14.0 Hz, 4.0 Hz, C2-H), 6.32 (2H, doublet, J = 9.0 Hz, aromatic H), 6.98 (1H, triplet, J = 9.0 Hz, aromatic H), 9.48 (2H, singlet, OH x 2, disappeared by the addition of D2O), 12.24 (1H, singlet, OH, disappeared by the addition of D2O). CD (c = 0.126, MeOH) [theta]<25o> (nm): -29000 (284), + 7800 (306), + 9400 (327). Yield 2.3 g.

According to an advantageous embodiment of this composition, said active substance is selected from the group constituted by wogonine, 2'-hydroxy-wogonine, baicaleine, neobaicaleine, oroxindine and baicaline.

According to a particular feature of this composition, the latter contains an extract of Scutellaria obtained by an extraction by solvent, preferably selected from the group consisting of a polar solvent, in particular an alcohol, water-alcohol or ethereal solution; of an apolar organic solvent such as n-hexane, benzene or a combination of the two.

According to a variant embodiment, an extraction is firstly effected with a polar organic solvent, followed by an extraction with an apolar organic solvent, in order to collect the fraction insoluble in the apolar solvent, as described in Planta Medica, Journal of Medicinal Plant Research, 1981, Vol. 43, pages 194-201.

The extraction procedure is described in Planta Medica at pages 194-200 as follows:

The extraction procedure is described in Planta Medica at pages 194-200 as follows:

The ether extract of roots of S. baicalensis was extracted with n-hexane. The n-hexane insoluble fraction, having anti-bacterial activity, was chromatographed on a column over silica gel by the procedure as shown in chart 1, yielding the antibacterial compound (VIII).

.elting points were determined on a Yamato Model MP-21 capillary and were ıncorrected. IR spectra were measured on a Shimazu IR-400. UV spectra were ɔbtained on a Shimazu MPS-5000. PMR spectra were recorded in DMSO-d6, CDCl3 and)2O on a Hitachi R-22 (90 MHz). TMS was used as an internal standard and :hemical shifts are reported in delta (ppm and Hz). Column chromatography was :arried out using silica gel 60 (70-230 mesh, ASTM. Merck) as adsorbent. ?re-coated TLC plates silica gel 60F254 (Merck) were used for TLC.
Extraction and Isolation of the Antibacterial Components

The crushed drug (10 kg) was extracted with 4 x 20 l of ether at room :emperature. The ether solution was concentrated to give a yellowish powder (140 g), which by treatment with n-hexane, was divided into soluble (40 g) and insoluble (100 g) fractions. The n-hexane insoluble extract (100 g) was :hromatographed on a column over silica gel to afford the compound as shown in FIG. 2.

According: to a variant embodiment of this composition, the latter is :haracterized in that the above-mentioned extract of Scutellaria alone or in ıixture with other compatible active substances, is introduced into the lipid)hase of the hydrated lipid lamellar phases or liposomes.

According to another variant embodiment of this composition, the latter is :haracterized in that the above-mentioned extract of Scutellaria alone or in ıixture with other compatible active substances, is introduced into the aqueous hase of the hydrated lipid lamellar phases or liposomes.

According to a particular embodiment, the extract of Scutellaria is an xtract selected from the group consisting of Scutellaria Baicalensis, of cutellaria Viscidula, or of Scutellaria Galericulata. According to an ıtageous embodiment, the above-mentioned extract of Scutellaria is an x..act of roots of Scutellaria Baicalensis Georgi, also called extract of Ogon.

According to a second aspect, the present invention also relates to a osmetic or pharmaceutical, particularly dermatological composition, with nti-inflammatory, anti-allergic or anti-ageing activity, characterized in hat it comprises a composition based on hydrated lipid lamellar phases or

that it comprises a composition based on hydrated lipid lamellar phases or liposomes, as defined hereinbefore.

The proportion by weight of the dry extract of Scutellaria or of any active substance obtained from such an extract or by chemical synthesis is included between 0.0001 and 2% relatively to the total weight of the composition; it is preferably included between 0.001 and 0.4% relatively to the total weight of the composition.

A crude extract of Ogon available on the market, particularly in 50% water-alcohol solution, may also be used in more practical manner, which extract may be used at a rate of 0.005 to 50%, and even better between 0.05 and 20% by weight with respect to the total weight of the composition.

Similarly, this pharmaceutical, particularly dermatological, or cosmetic composition may, according to a first variant, be characterized in that the above-mentioned extract of Scutellaria, or any active substance extracted therefrom, alone or mixed with other compatible active substances, is introduced in the lipid phase of the hydrated lipid lamellar phases or liposomes, whilst, according to another variant, such introduction may be effected in the aqueous phase of the hydrated lipid lamellar phases or liposomes.

In the present description and Claims, the term "lipid" in the expression "lipid lamellar phase" covers all the substances comprising a so-called fatty carbon chain, generally more than 5 carbon atoms.

According to the invention, amphiphilic lipids are used, i.e. constituted by molecules presenting a hydrophilic group which may equally well be ionic or non-ionic and a lipophilic group, such amphiphilic lipids being capable of forming lipid lamellar phases in the presence of an aqueous phase. The following may be cited in particular among these lipids: phospholipids, phosphoaminolipids, glycolipids, polyoxyethylene fatty alcohols, possibly polyoxyethylene polyol esters. Such substances are for example constituted by an egg or soya lecithin, a phosphatidylserine, a sphyngomyelin, a cerebroside or an oxyethylene polyglycerol stereate.

Other purposes, characteristics and advantages of the invention will clearly appear on reading the following explicative description made with reference to several Examples of the invention given simply by way of illustration and which should therefore in no way limit the scope of the invention. In the Examples, the percentages are given by weight unless indicated to the contrary.

DRWDESC:
BRIEF DESCRIPTION OF DRAWINGS

DRWDESC:
BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a histogram of antiphlogistic activity based on the results of Table II. The height of the bars represent the magnitude of the inflammatory reaction provoked by the croton oil.

FIG. 2 is a flow chart of the isolation of antibacterial substance (VIII), ...in (I), Skullcapflavone II (IV) and Baicalein (V) from the roots of Scutellaria Baicalensis.

DETDESC:

EXAMPLE 1
A-Preparation of a Composition in the Form of an Advantageously Homogenized Liposome Suspension

The extract of Ogon used is an extract of Ogon obtained from Scutellaria Baicalensis Georgi root marketed by the Japanese firm Ichimaru Pharcos Co. Limited, constituted here by batch No. IT134, called "Woogon extract-E", and which is in the form of a 50% vol/vol water-alcohol solution in ethanol, having a density of 0.931, a residue content after evaporation of 1.38% by weight/volume, a content of baicaline of 0.16% by weight/volume and a very low content of baicaleine.

This extract of Ogon (also called Ougon or Woogon in the art) may be evaporated to dryness, or, in more practical manner, used as such for preparing a composition in the form of liposome suspension, in the following manner:

composition

extract of Ogon (Woogon extract No. IT134, 50% water-alcohol solution in ethanol)	0.5 g
bidistilled water	47.5 g
lecithin of soya	2.0 g

This composition prepared the following manner:

The Ogon extract is firstly added in the bidistilled water, with stirring, then the soya lecithin is dispersed in this aqueous solution.

This solution is homogenized with continued stirring for about 2 hrs.

This solution is homogenized with continued stirring for about 2 hrs.

Homogeneization is preferably carried out by ultra-sounds, by effecting a sonication for 10 mins. at 100 W, which makes it possible to obtain a mean liposome dimension of the order of 106.7 nm +/- 0.5 nm.

Instead of an ultra-sound homogeneization, a homogeneization under pressure m[illegible]be carried out, for example in accordance with the process described in U.S. P[illegible] No. 4,621,023.

It will be observed that various dilutions may be made by modifying the quantity of extract added at the start or by increasing the volume of the solution of dispersion, which constitutes an easy process for preparing various concentrations of extract.

In the absence of a dilution, 50 g of homogenized suspension, corresponding to about 50 ml, are obtained after this step A.

B-Preparation of a Composition of Homogenized Liposomes in the Form of Gel

This homogenized suspension may be gelled by mixture with a gel, such as a vinyl polymer gel, in particular marketed under the Trademark Carbopol Registered TM 940.

To prepare this gel in conventional manner, 0.5 g of Carbopol Registered TM 940 may for example be dispersed in 50 g of water in the presence of a conservation agent and a conventional chelating agent, then, after swelling, triethanolamine may preferably be used for neutralizing to pH 7.5.

In this way, 50 ml of said gel are added to the 50 g or milliliters of homogenized suspension obtained in step A hereinabove, in order to obtain a total volume of about 100 ml.

In this gelled composition, the concentration of dry extract of Ogon is about 0,069% and the concentration of lecithin is 2%.

This composition thus gelled, referenced CI.1, will be used in the activity trials given hereinbelow in the present description.

EXAMPLE 2

Composition of Liposomes Containing Extract of Ogon in the Lipid, Possibly Gelled Phase

1.0 g of Ogon extract of Example 1 is taken, which is dissolved in 50 cm<3 > of chloroform.

The substance is evaporated to dryness under reduced pressure in a rotary evaporator at a temperature of the order of 56o C.

The residue deposited on the wall of the rotary flask is taken up with 10 ml of methanol.

2 g of soya lecithin as well as 50 ml of chloroform are added.

The whole solution is evaporated under reduced pressure in the same rotary flask at a temperature of about 56o C. in order to obtain a film which is deposited on the wall of the rotary flask.

This film is then taken up with 48.0 g of water.

Stirring is effected for 3 hrs. with the aid of a magnetic stirrer in order to obtain a suspension of liposomes containing the extract of Ogon at least in part in the lipid phase.

The liposomes may be homogenized by sonication for 10 mins. at 100 W in an ice bath in order to obtain a suspension of homogenized liposomes.

This solution may possibly be gelled in the same manner as in Example 1, in order to obtain a gelled composition having a concentration of dry extract of Ogon of about 0.138% by weight/volume and a concentration of lecithin of 2%.

EXAMPLE 3

Baicaleine in Liposomes

The procedure is the same as in Example 2, except that 0.1 g of baicaleine is used in place of the extract of Ogon.

EXAMPLE 4

Comparative Compositions

C-A

0.5 g of the extract of Ogon of Example 1 is taken which is added to 49.5 g of bidistilled water and a mixture is made with stirring for some minutes.

5 g of 1% Carbopol Registered TM 940 gel prepared as described in Example 1B, are then added so as to obtain a gelled composition for comparison referenced C.P. No. 1.

C-B

A control comparative composition referenced C.P No. 2 is also prepared by mixing 50 g of bidistilled water and 50 g of 1% Carbopol 940 gel prepared as described in Example 1.

escribed in Example 1.
-C

Another liposomal comparative composition is prepared in the manner indicated
n Example 1, without active substance, i.e. no extract of Ogon is employed.
his composition is therefore formed by 2 g of soya lecithin, 47.5 g of
idistilled water, then the Suspension which was homogenized with ultrasounds as
ated in Example 1A is gelled as indicated in Example 1B in order to furnish
mparative composition referenced C.P No. 3.

XAMPLE 5

emonstration of the Anti-Allergic and Anti-Inflammatory Activity of the
ompositions According to the Invention

The use of the composition of Example 1 as pharmaceutical, particularly
ermatological, or cosmetic composition is checked by carrying out the following
n vivo experiments:

1-Demonstration of the anti-allergic activity

The anti-allergic activity is tested by the DNCB
chloro-1-dinitro-2,4-benzene) test in the guinea pig.

To that end, 5 batches of 10 guinea pigs having substantially the same weight
nd presenting no detectable sign of allergy, are formed.

The 50 guinea pigs are sensitized with DNCB by an intradermic injection of
NCB at 0.2% by weight. One week afterwards, they receive a topical application
the same solution of. DNCB at 0.2%.

The guinea pigs are then left to rest for 12 days.

After this period, the allergic reaction is again provoked by a fresh patch
pplication of a solution of DNCB at 0.02%.

In order to test the anti-allergic activity of the composition according to
e invention of Example No. 1 (batch No. 1) in comparison with the other
mparative compositions indicated hereainafter (batch No. 2 to batch No. 5),
uinea pigs had received in application, 1 hour before the patch
plication, respectively:

for batch No. 1: 1 ml of extract of Ogon in liposome (CI 1 of Example 1)
gon in liposomes in gel)

for batch No. 2: 1 ml of extract of Ogon at 0.5% in gel (CP No. 1 of Example

for batch No. 2: 1 ml of extract of Ogon at 0.5% in gel (CP No. 1 of Example 4-A) (Ogon in gel)

for batch No. 3: 1 ml of gel (C.P No. 2 of Example 4-B) (gel)

for batch No. 4: 1 ml of "empty" liposomes in gel (C.P No. 3 of Example 4-C)

or batch No. 5: no application (control batch).

The intensity of the allergic reaction of the animals is noted from 0 to 5, the mark 5 being attributed to the most intense reaction observed.

Table I hereinbelow indicates the number of animals for each mark attributed.

TABLE I

	0	1	2	3	4	5	TOTAL
1: C.I 1: ogon in liposomes in gel	1	5	2	1	1	-	16
2: C 1: ogon in gel	-	4	2	1	3	-	23
3: CP 2: gel	1	4	1	2	1	1	21
4: CP 3: "empty" liposomes in gel	2	2	2	2	2	-	20
5: control	-	-	-	2	6	2	40

The results shown in Table I very clearly show that the allergic reactions are on average much less intense in the guinea pigs previously treated with the suspension of liposomes containing the extract of Ogon (batch No. 1), with respect to those observed in the animals of batches No. 2 to No. 5 receiving compositions taken by way of comparison (batch No. 2 to batch No. 4) or without any application (batch No. 5).

Moreover, it may also be observed that the extract of Ogon appears clearly active in liposomes with respect to the free form in gel (batch No. 2).

This test therefore incontestably demonstrates the intense anti-allergic activity obtained by the incorporation of Ogon extract in hydrated lipid lamellar phases or liposomes made according to the invention.

2 -Demonstration of the anti-inflammatory activity

2 -Demonstration of the anti-inflammatory activity

The anti-inflammatory activity of the composition according to the invention is demonstrated by a croton oil test, made in accordance with the TONELLI method in the Journal Endocrinology, 1965, vol. 77, pages 624-634, made on the albino mouse in accordance with the following procedure:

The mice are distributed in seven batches of 8 mice, each batch being treated with a given product:

Batch No. 1 is treated with Ogon in liposomes,

Batch No. 2 is treated with empty liposomes in gel,

Batch No. 3 is treated with Ogon in gel,

Batch No. 4 is treated with gel alone,

Batch No. 5 is treated with Dectancyl,

Batch No. 6 is a control batch on which only croton oil is applied,

Batch No. 7 is another control batch which does not undergo any application, therefore without croton oil.

0.1 ml of the product to be tested is applied on the right ear 3 hrs., 2 hrs. and 1 hr. before the application of 0.05 ml of croton oil at 0.2% in acetone.

In order to avoid artifact phenomena at the level of absorption of the products, the oily excipients are proscribed in this study.

5 1/2 hrs. later, the animals are sacrificed.

The treated ear is taken and weighed on precision scales (mettler).

The average of the weights is effected for each batch.

The average of the control weights will then be subtracted from the results obtained in the other batches, in order to obtain the values of increase in the weights of ears, with respect to the normal animals.

The percentage of protection of the tested products is then made by bringing the croton oil batch to 100%.

Table II gives the individual results.

Table III presents the statistical analysis by t of Student.

The results of Table II are shown in the form of a histogram in accompanying FIG. 1; the height of the bars represents the magnitude of the inflammatory reaction provoked by the croton oil applied in accordance with the procedure indicated hereinbefore.

It may be observed from these results of tests that the edema provoked by croton oil (measured by weighing the ear) is not significantly reduced by the products applied, except for the extract of Ogon incorporated in liposomes in accordance with the present invention, and Dectancyl.

The activity of Dectancyl is significantly greater than the activity of the other products.

The extract of Ogon in gel, as well as the gel alone or the "empty" liposomes in gel, contributes no anti-phlogistic activity.

In addition, it may be observed that the extract of Ogon in liposomes according to the invention contributes an anti-phlogistic activity of 69.6%, which is remarkable and completely unexpected for a man skilled in the art.

TABLE II

	Composition tested						
	* Ogon in liposomes (ex. 1, CI1)	* Empty liposomes in gel (ex. 3-O (CP3)	* Ogon in gel (CP1) (ex. 3-A)	* Gel alone (ex. 3-B) (CP2)	* Dectancyl	* Control (only croton oil)	Control (without croton oil)
1	138	161	209	278	121	183	134
2	147	167	194	195	126	192	140
3	146	255	187	190	123	224	130
4	160	190	210	240	121	170	122
5	157	166	208	172	100	188	140
6	165	172	214	202	108	216	135
7	167	175	190	185	133	200	133

7	167	175	190	185	133	200	133
8	156	160	194	181	*	238	138
AVERAGE	154.5	180.7	200.7	205.3	118.8	201.4	134
STANDARD DEVIATION	10.04	31.4	10.5	35.7	11.2	22.8	5.9
AVERAGE PRODUCT MINUS AVERAGE CONTROL	20.5	46.7	66.7	71.3	- 15.2	67.4	
STANDARD DEVIATION	15.9	36.3	16.4	41.6	17.1	28.7	
Brought to 100%	30.4	69.3	98.9	105.7	- 22.5	100	
PROTECTION	69.6%	30.7%	1.1%	- 5.7%	122.5		

TABLE III

	OGON IN LIPOSOMES GEL (Ex. 1) (CI1)	EMPTY LIPOSOMES IN GEL (Ex. 3-C) (CP3)	* GEL ALONE OGON IN GEL (Ex. 3-A) (CP1)	(Ex. 3-B) (CP2)	* DECTANCYL	* CONTROL (without croton oil)
OGON IN LIPO-SOMES IN GEL (Ex. 1) (CI1)	*	> *	> ***	> **	> ***	> ***
EMPTY LIPOSOMES IN GEL (Ex. 3-C) (CP3)	*	*	NS	NS	> ***	NS
OGON IN GEL (Ex. 3-A) (CP1)	*	*	*	NS	> ***	NS
ALONE (Ex. 3-B) (CP2)	*	*	*	*	> ***	NS
DECTANCYL	*	*	*	*	*	> ***

nt = 2,19 -

nt = 2,19 -

n5% = 2,14 -

n* = 5% -

n** = 1% -

n*** = 0,1% -

Various examples of dermatological, dermocosmetic compositions are given hereinafter:

EXAMPLE 6

Cream for Sensitive Skins

A mixture of suspension of liposomes containing Ogon with an oil-in-water type emulsion is made in the following proportions:

Composition of liposomes prepared in accordance with Example 1 with 0.2% dry extract of Ogon	25	g
Emulsified oil-in-water excipient	qsp 100	

A daily or twice-daily application is made in cold, dry weather.

EXAMPLE 7

Cream for Care Around the Eyes

A mixture of suspension of liposomes containing Ogon with an oil-in-water type emulsion is made in the following proportions:

Composition of liposomes prepared in accordance with Example 2 with 0.15% dry extract of Ogon	30	g
Emulsified oil-in-water excipient	qsp 100	

A daily application is made on the lower and upper eyelids.

EXAMPLE 8

Milk

A mixture of suspension of liposomes containing Ogon with an oil-in-water type emulsion is made in the following proportions:

Composition of liposomes prepared in accordance with Example 2 with	30	g

in accordance with Example 2 with
0.15% dry extract of Ogon
Emulsified oil-in-water excipient qsp 100

XAMPLE 9
rotective Gel for Skins Subject to Allergies

Composition of liposomes prepared in accordance with Example 1 with 0.2% dry extract of Ogon 50 g
Gelled excipient qsp 100

Use in daily local application to avoid the appearance of allergic reaction r to reduce the magnitude thereof.

XAMPLE 10
ascara for Sensitive Eyelids

Composition of liposomes prepared in accordance with Example 2 with 0.15% dry extract of Ogon 20 g
Gelled oil-in-water emulsion laden with pigments qsp 100

KAMPLE 11
omposition for Masking Circles (Around Eyes) Intended for Sensitive Skins

Composition of liposomes prepared in accordance with Example 1 with 0.2% dry extract of Ogon 10 g
Oil-in-water emulsion laden with pigments qsp 100

KAMPLE 12
undation for Sensitive Skins

Composition of liposomes prepared in accordance with Example 1 with 0.1% dry extract of Ogon 20 g
Oil-in-water emulsion laden with pigments qsp 100

EXAMPLE 13
Anti-Allergic and Anti-Inflammatory Composition

Composition of liposomes prepared in accordance with Example 3 with 0.1% of baicaleine	20	g
Oil-in-water emulsion	qsp 100	

EXAMPLE 14
Day Cream for Delaying Ageing of the Skin

Composition of liposomes prepared in accordance with Example 1 with 0.3 dry extract of Ogon	20	g
Emulsified oil-in-water excipient	qsp 100	

Daily application in the morning on those parts of the body exposed to daylight.

CLAIMS: I claim:

[*1] 1. Hydrated lipidic lamellar phases or liposomes consisting essentially of an extract of Scutellaria as the active ingredient, said extract being obtained by extraction with a solvent selected from the group consisting of a polar organic solvent, a polar water and organic solvent mixture, and an apolar organic solvent.

[*2] 2. Hydrated lipidic lamellar phases or liposomes of claim 1, wherein the Scutellaria extract is obtained by extraction of Scutellaria roots with a polar organic solvent.

[*3] 3. Hydrated lipidic lamellar phases or liposomes of claim 1, wherein the Scutellaria extract is obtained by extraction of Scutellaria roots with a water-alcohol solution.

[*4] 4. Hydrated lipidic lamellar phases or liposomes of claim 1, wherein the Scutellaria extract is obtained by extraction of Scutellaria roots with an apolar organic solvent.

[*5] 5. Hydrated lipidic lamellar phases or liposomes of claim 1, wherein the Scutellaria extract is obtained by extraction of Scutellaria roots with a 50% vol/vol water-ethanol solution.

50% vol/vol water-ethanol solution.

[*6] 6. Hydrated lipidic lamellar phases or liposomes of claim 1, wherein the Scutellaria is selected from the group consisting of Scutellaria Baicalensis, Scutellaria Viscidula and Scutellaria Galericulata.

[*7] 7. Hydrated lipidic lamellar phases or liposomes of claim 1, wherein the Scutellaria extract is an extract of Scutellaria Baicalensis Georgi.

[*8] 8. Hydrated lipidic lamellar phases or liposomes of claim 7, wherein the Scutellaria extract is an extract of roots of Scutellaria Baicalensis Georgi.

[*9] 9. Hydrated lipidic lamellar phases or liposomes of claim 8, wherein the extract of roots of Scutellaria Baicalensis Georgi is an extract obtained by extraction with a 50% vol/vol water-ethanol solution.

[*10] 10. Hydrated lipidic lamellar phases or liposomes consisting essentially of an extract of Scutellaria as the active ingredient thereof having anti-inflammatory, anti-allergic or anti-aging activity, said extract being obtained by extraction with a solvent selected from the group consisting of a polar organic solvent, a polar water and organic solvent mixture, and an apolar organic solvent.

[*11] 11. Hydrated lipidic lamellar phases or liposomes of claim 10, wherein the Scutellaria extract is obtained by extraction of Scutellaria roots with a polar organic solvent.

[*12] 12. Hydrated lipidic lamellar phases or liposomes of claim 10, wherein the Scutellaria extract is obtained by extraction of Scutellaria roots with a water-alcohol solution.

[*13] 13. Hydrated lipidic lamellar phases or liposomes of claim 10, wherein the Scutellaria extract is obtained by extraction of Scutellaria roots with an apolar organic solvent.

[*14] 14. Hydrated lipidic lamellar phases or liposomes of claim 10, wherein the Scutellaria extract is obtained by extraction of Scutellaria roots with a 50% vol/vol water-ethanol solution.

[*15] 15. Hydrated lipidic lamellar phases or liposomes of claim 10, wherein the Scutellaria is selected from the group consisting of Scutellaria Baicalensis, Scutellaria Viscidula and Scutellaria Galericulata.

[*16] 16. Hydrated lipidic lamellar phases or liposomes of claim 10, wherein the Scutellaria extract is an extract of Scutellaria Baicalensis Georgi.

[*17] 17. Hydrated lipidic lamellar phases or liposomes of claim 16, wherein the Scutellaria extract is an extract of roots of Scutellaria Baicalensis Georgi.

[*18] 18. Hydrated lipidic lamellar phases or liposomes of claim 17, wherein the extract of roots of Scutellaria Baicalensis Georgi is an extract obtained by extraction with a 50% vol/vol water-ethanol solution.

*Pat. No. 5908628, **

5,908,628

Jun. 1, 1999

Compositions with analgesic, antipyretic and antiinflammatory properties

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INT-CL: [6] A01N 65#00; A61K 35#24

US-CL: 424#195.1; 424#537; 514#825; 514#886; 514#916

CL: 424;514

SEARCH-FLD: 424#195.1, 537, 520, 489; 514#916, 886, 887, 825

REF-CITED:

U.S. PATENT DOCUMENTS

5,417,979	5/1995	*	Fan et al.	424#451
5,466,452	11/1995	*	Whittle	424#195.1
5,595,743	1/1997	*	Wu	424#195.1
5,627,195	5/1997	*	Hu	514#321

FOREIGN PATENT DOCUMENTS

1101577	4/1995	*	China
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Koo Dong Ham et al., J. of the Pharmaceutical Society of Korea, vol. 19(3), pp. 129-155, 1975.

PRIM-EXMR: Prats, Francisco

ASST-EXMR: Vidovich, Kristin K.

LEGAL-REP: Morgan, Lewis & Bockius LLP

CORE TERMS: composition, ditto, rat, plant, root, radix, dried, inflammation, sample, ingredient, mice, herbal, mixture, aspirin, herb, body weight, injection, fever, silkworm, stemona, resultant, paw, siegesbeckia, ethyl alcohol, gastrogavage, inhibitory, chloroform, capsule, pain, rheumatoid arthritis

ABST:

000024

The present invention provides compositions comprising talc, silkworm excrement, and ingredients of plants of species of the genera Stephania, Coix, Pinellia, Prunus, Phellodendron, Sophora, Tetrapanax, Stemona, Glycyrrhiza, Tripterygium, Forsythia and Siegesbeckia, wherein such compositions have analgesic, antipyretic, and antiinflammatory properties. The present invention also provides methods of using such compositions for treating various diseases, including osteoarthritis and rheumatoid arthritis.

NO-OF-CLAIMS: 17

EXMPL-CLAIM: 1

NO-OF-FIGURES: 0

NO-DRWNG-PP: 0

SUM:

FIELD OF THE INVENTION

The present invention pertains, in general, to the field of therapeutic compounds for the treatment of pain, fever and inflammation. In particular, the present invention pertains to compositions comprising talc, silkworm excrement and various herbs, wherein such compositions are useful for the treatment of rheumatoid and/or arthritic conditions.

BACKGROUND OF THE INVENTION

All publications and patent applications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Rheumatism refers to any of several pathological conditions of the muscles, tendons, joints, bones, or nerves, characterized by discomfort and disability. It is estimated that over 100 rheumatic diseases affect the joints and other connective tissues of animals.

Arthritis generally refers to the inflammation of a joint or joints which results in pain and swelling. The two most common forms of arthritis are osteoarthritis and rheumatoid arthritis. Osteoarthritis is characterized by chronic degeneration of the cartilage of the joints, mainly in older persons. Rheumatoid arthritis, sometimes called arthritis deformans, is a chronic and progressive systemic disease, especially common in women, characterized by stiffness, swelling and inflammation of the joints and sometimes leading to deformity and permanent disability. Sufferers of rheumatoid arthritis may also have general symptoms of fatigue, weakness, and loss of appetite. While there is no cure, these diseases can sometimes be managed by lifestyle and diet changes.

Many other diseases also cause pain, inflammation and fevers. Rheumatic fever is an acute inflammatory disease occurring during recovery from infection with group A streptococci, having an onset marked by fever and joint pain. It is associated with polyarthritis, Sydenham's chorea, and endocarditis, and is frequently followed by scarring of the heart valves. Lupus erythematosus, also known as systemic lupus erythematosus, is a chronic disease of unknown origin characterized by red, scaly lesions or patches on the face and upper portion of the trunk. Erythema nodosum is a skin disease associated with joint pain, fever, hypersensitivity, or infection, and characterized by small, painful, pink to blue nodules under the skin and on the shins that tend to recur. Gout is an inherited disorder of uric-acid metabolism occurring predominantly in men, characterized by painful inflammation of the joints, especially of the feet and hands, and arthritic attacks resulting from elevated levels of

uric acid in the blood and the deposition of urate crystals around the joints. The condition can become chronic and result in deformity.

When diet and lifestyle changes are not sufficient to alleviate the symptoms of rheumatism and rheumatoid-like diseases, pharmaceuticals are often used for relief from the resultant pain, discomfort, and fever. The antiinflammatory, analgesic and antipyretic agents and drugs often employed for this purpose are a heterogeneous group of compounds; often chemically unrelated, which nevertheless share certain therapeutic actions and side effects. For a comprehensive discussion of such drags see Insel, P. A., Analgesic-Antipyretic and Antiinflammatory Agents and Drugs Employed in the Treatment of Gout, In Goodman & Gilman's The Pharmacological Basis of Therapeutics, Ninth Edition, Chapter 27 (1996).

Herbal Medicines

It is estimated that approximately 50 percent of the thousands of drugs commonly used and prescribed today are either derived from a plant source or contain chemical imitations of a plant compound (Mindell, E. R., Earl Mindell's Herb Bible, A Fireside Book (1992)). Currently, a number of medicinal formulations contain herbal components or extracts from herbs. Technically speaking an herb is a small, non-woody (i.e., fleshy stemmed), annual or perennial seed-bearing plant in which all the aerial parts die back at the end of each growing season. As the word is more generally used and as it is used herein, an herb is any plant or plant part which has a medicinal use. Thus, the term herb is also generally used to refer to the seeds, leaves, stems, flowers, roots, berries, bark, or any other plant parts that are used for healing.

Herbal medicines have been used for treating various diseases of humans and animals in many different countries for a very long period of time (see, e.g., Kessler et al., The Doctor's Complete Guide to Healing Medicines, Berkley Health/Reference Books (1996); Mindell, supra). Herbal medications are available in many forms, including capsules, tablets, or coated tablets; pellets; extracts or tinctures; powders; fresh or dried plants or plant parts; prepared teas; juices; creams and ointments; essential oils; or, as combinations of any of these forms. Herbal medicines are administered by any one of various methods, including orally, rectally, parenterally, enterally, transdermally, intravenously, via feeding tubes, and topically.

The bark of the willow tree has been used to treat fever since the mid-eighteenth century in England. The active ingredient in willow bark is a bitter glycoside called salicin, which on hydrolysis yields glucose and salicylic alcohol. Aspirin (acetylsalicylic acid) and aspirin-like drugs (e.g., ibuprofen), all of which are often called nonsteroidal antiinflammatory drugs (NSAIDs), are frequently used to treat pain, fever, and inflammation. Meadowsweet is another herb that contains salicylates. Treatment of arthritic and arthritic-like symptoms with willow bark or meadowsweet requires the consumption of prohibitively large quantities of herbal teas made from these plants. The entire Populus species (i.e., poplar trees and shrubs) also contains salicylate precursors and poplar-buds have been used in antiinflammatory, antipyretic and analgesic medications.

While preliminary evidence suggests that joint inflammation may be reduced by the intake of plants which contain gamma-linolenic acid (e.g., black currant, borage, evening primrose), relief using these plants also requires the intake of large amounts of plant material. The alkaloid colchicine is extracted from the corm and the seeds of autumn crocus (Colchicum autumnale) and used in either tablet form or intravenously for patients with gout. Colchicine is also used to treat familial Mediterranean fever. However, as little as 7 mg of colchicine has been found to be fatal, although the normal fatal dose is 65 mg. European goldenrod (Solidago virgaurea) has been used to treat arthritis, kidney inflammation, and as a headache remedy for treating flu, sore throat, malaria and measles. Modern research has found that licorice (Glycyrrhiza glabra), which contains a medically active terpene

component, can reduce arthritic activity. However, the cortisone-like component of the saponin like glycoside glycyrrhizin causes dangerous side effects, including abnormal heart action and kidney failure, triggered by potassium depletion. While alfalfa, black cohosh, blue-green algae, bog bean, burdock root, celery seeds, chaparral, comfrey, dandelion, devil's claw, feverfew, fresh ginger, juniper, mustard, parsley, sassafras, valerian, wormwood and yucca have all been reputed to bring relief of arthritis, there is little or no scientific evidence to support such assertions. For a more complete discussion of herbal-based medicines see Mindell, supra; Culpeper's Complete Herbal, W. Foulsham & Co., Ltd. (originally published in the mid 1600's); and, Rodale's Illustrated Encyclopedia of Herbs, Rodale Press (1987).

U.S. Patents have been issued for herbal medicinals used for the treatment of various diseases and other health-related problems afflicting humans and animals. For example, U.S. Pat. No. 5,417,979 discloses a composition comprising a mixture of herbs, including species of Stephania and Glycyrrhiza, as well as their extracts, which is used as an appetite stimulant and for the treatment of pain. Herbal compositions which include Glycyrrhiza uralensis have been found useful for treating eczema, psoriasis, pruritis and inflammatory reactions of the skin (U.S. Pat. No. 5,466,452). U.S. Pat. No. 5,595,743 discloses various herbal compositions which include licorice extract (Glycyrrhiza) and siegesbeckia, sophora, stemona and tetrandra herbs used for the treatment of various mammalian diseases, including inflammation and rheumatoid arthritis. Ocular inflammation can be treated with a pharmaceutical composition containing the plant alkaloid tetrandrine (U.S. Pat. No. 5,627,195). U.S. Pat. No. 5,683,697 discloses a pharmaceutical composition having anti-inflammatory, anti-fever, expectorant or anti-tussive action, wherein the composition includes plant parts from the species Melia, Angepica, Dendrobium, Impatiens, Citrus, Loranthus, Celosia, Cynanchum and Glehnia. An herbal formulation comprising extracts of the roots, rhizomes, and/or vegetation of Alphinia, Smilax, Tinospora, Tribulus, Withania and Zingiber has been found to reduce or alleviate the symptoms associated with rheumatoid arthritis, osteoarthritis, reactive arthritis and for reducing the production of proinflammatory cytokines (U.S. Pat. No. 5,683,698).

Based on the foregoing, there currently exists a need for antiinflammatory, analgesic and antipyretic herbal-based therapeutics which have low toxicity and few side effects. For example, there is a need for non-aspirin therapeutics for the treatment of fever, pain and inflammation associated with rheumatoid arthritis and osteoarthritis. The novel compositions of the present invention fulfill those requirements.

SUMMARY OF THE INVENTION

This invention comprises compositions for reducing inflammation, pain, and fever in a mammal, as well as methods of using such compositions in the treatment of these symptoms in animals.

The compositions of the present invention have analgesic, antipyretic, and antiinflammatory properties. More specifically, the compositions of the present invention can be used to alleviate symptoms associated with rheumatism and/or arthritis, especially those associated with rheumatoid arthritis and osteoarthritis.

The present invention provides compositions comprising talc, silkworm excrement, and ingredients of plants of species of the genera Stephania, Coix, Pinellia, Prunus, Phellodendron, Sophora, Tefrapanax, Stemona, Glycyrrhiza, Tripterygium, Forsythia and Siegesbeckia, wherein such compositions have analgesic, antipyretic, and antiinflammatory properties.

More specifically, the present invention comprises talc, silkworm excrement, and an extract from the seeds, roots, tubers, rhizomes, and/or vegetation of Stephania, Coix, Pinellia, Prunus, Phellodendron, Sophora, Tetrapanax, Stemona, Glycyrrhiza, Tripterygium, Forsythia

and Siegesbeckia.

Even more specifically, the present invention provides compositions comprising talc, silkworm excrement and ingredients of plant species, wherein the plant ingredients include the root of Stephania, kernels of Coix, rhizomes of Pinellia, seeds of Prunus, bark of Phellodendron, roots of Sophora, stem of Tetrapanax, root tubers of Stemona, roots and/or rhizomes of Glycyrrhiza and Tripterygium, fruit of Forsythia and the above-ground parts of Siegesbeckia.

The present invention provides compositions comprising the active ingredients tetrandine, sophocarpidine, hydrochloric phellodendrine, triptolide and saponin, wherein the compositions have analgesic, antipyretic, and antiinflammatory properties.

One skilled in the art can easily make any necessary adjustments in accordance with the necessities of the particular situation. Further objects and advantages of the present invention will be clear from the description and examples which follow.

DETAILED DESCRIPTION OF THE INVENTION

I. GENERAL DESCRIPTION

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described.

The current inventions in this application are in the fields of herbal-based pharmaceuticals and methods of using such medicines for the treatment of fever, pain and inflammation.

The compositions and methods of the present invention find particular application in the treatment of symptoms associated with various rheumatoid and rheumatoid-like diseases, especially osteoarthritis and rheumatoid arthritis.

Utilizing the results provided below, a skilled artisan can readily practice and develop the diagnostic, screening and therapeutic methods outlined herein and in the claims.

During a long-term rheumatoid disease research effort, the inventor of the present invention formulated numerous herbal-based medicinals and assessed their effectiveness in treating the fever, pain and inflammation associated with the disease. The compositions of the present invention were developed as a result of thousands of clinical assessments using various herbal-based medicines to treat rheumatoid symptoms. As discussed herein, the compositions of the present invention can be used instead of hormonal medicines or aspirin-based drugs to control the symptoms of rheumatoid arthritis during its active stages and to prevent articular deformity. The total effective rate of the composition approaches 97% in human clinical trials.

The composition of this invention comprises talc ((Mg3(Si4O10)(OH)2), a silkworm excrement, and ingredients of plants of species of the genera Stephania, Coix, Pinellia, Prunus, Phellodendron, Sophora, Tetrapanax, Stemona, Glycyrrhiza, Tripterygium, Forsythia and Siegesbeckia. The individual components of the composition are described in greater detail in the following sections.

The Detailed Description and Examples provide detailed scientific results that can be used by a skilled artisan to prepare and administer the compositions of the present invention. The

description of the present invention provided herein has been given for clearness of understanding only and no unnecessary limitations should be understood therefrom as modifications will be obvious to those skilled in the art.

II. COMPONENTS OF THE COMPOSITION

The composition of the present invention comprises talc, silkworm excrement and the dried plant parts of twelve different Chinese herbs. Each individual component is more clearly defined as set forth in the descriptions which follow.

A. Pulvis talci (talc)

General Description

The pulvis talci component of the composition is made with refined, purified, smashed/pulverized, and dried ore of talcum ((Mg3(Si4O10)(OH)2). The dried ore of talcum utilized in the present invention was obtained from the Shandong and Jiangxi provinces of China.

Characteristics

White or nearly white color; fine texture; non-sandy powder, satiny feel, odorless, tasteless, and does not dissolve in water, dilute mineral acid, or alkali hydroxide.

pH test.

10 g of the substance is added to 50 ml of water, boiled for 30 minutes during which the evaporated moisture is replaced, then filtered. The filtrate shows neutral reaction upon conducting a litmus paper test.

Water-solubility.

50 g of the substance is added to 30 ml of the water, boiled for 30 minutes during which the evaporated moisture is replaced, cooled, filtered, and the residue from filtering is cleaned with 5 ml of water. The cleaning solution is combined with the filtrate and steam dried for 1 hour at 105o C. The residue will be 5 mg (0.1%) or less.

Acid solubility.

1 g of the substance is added to 20 ml of dilute hydrochloric acid, immersed for 15 minutes at 50o C., then filtered. 1 ml of dilute acid is added to 10 ml of the filtrate which is steamed dried and heated until constant weight is achieved. The residue will be 7.5 mg (1.5%) or less.

Ferric salt test.

10 g of the substance is added to 30 ml of water, heated and boiled for 30 minutes, the evaporated moisture is replaced, and the resultant mixture is cooled and filtered. 1 ml of dilute hydrochloric acid and potassium ferrocyandide are consecutively added to the filtrate. The resultant product will never show a blue color instantly.

Ignition weight:

The substance is heated until achieving a constant weight at 600-700o C. The total loss of weight in the resultant product is 5.0% or less.

Storage

The resulting substance is stored in air-proof containers.

B. Excrementum bombycis (silkworm excrement)

General Description

The excrementum bombycis component is made from the dried, solid feces excreted by Bombyx mori L., commonly known as the silkworm (family: Bombycidae). The silkworm is a moth caterpillar creamy white in color and approximately 7.6 cm long. The silkworm feeds chiefly on mulberry leaves and spins its cocoon from a silk fiber secreted by glands in its body. Either domesticated or wild silkworms can be used for preparing the excrementum bombycis used in the present invention. The silkworms utilized in the examples of the present invention were obtained from the Sichuan, Jiangsu, and Zhejiang provinces of China.

Chemical Composition of Sample

Organic substance:	83.77-90.44%
Ash:	9.56-16.23%
Nitrogen content:	1.91-3.60%
Chlorophyl:	1.6-2.4%
Pure chlorophyl:	13.9%
Saponifying substance:	48.9%
Phytol:	0.25-0.29%
Non-Saponifying substance:	beta -sitosterol 1.5% cholesterol ergosterol lupeol beta -glucoscillaren free amino acid carotene.

Extraction Process

Excrementum bombycis is placed into an extractor, 70% of ethanol is added, and the resultant mixture is heated under reflux. Ethanol is recovered and condensed under low-temperature and decompression until the specific density reaches 1.38 (thermal assay). The extract is then collected by vacuum drying.

Phototoxicity

Photoxicity tests conducted using excrementum bombycis prepared from the excrement of silkworms fed an herbal diet demonstrate a slight, acceptable, phototoxic reaction. The phototoxicity tests are conducted based on the phototoxicity of sodium trichorophyllin and polymers thereof.

C. Herbal Components

The herbal components of the composition of the present invention are each provided herein. While the following examples provide preferred species and preferred plant parts to be used for each herbal component, as well as the geographic origin of the herbs used in the examples, one skilled in the art readily recognizes that alternative plant species and alternative plant parts, as well as alternative geographic sources for the plants, will satisfy the requirements of the composition. If herbs of an alternative species and/or plant part

and/or plant origin were used to prepare the composition, then one skilled in the art could easily make any necessary adjustments in the preparation of the compostion to account for the lower or higher concentrations of any particular active ingredient.

Each of the following herbal components except leigongteng are included in the official medicaments described in the Chinese Pharmacopoeia (1990), which is herein incorporated by reference in its entirety.

1. Cortex phellodendri (Phellodendron bark)

General Description

The dried bark of Phellodendron chinense Schneid, or Phellodendron amurense Rupr. (family-Rutaceae) is used in the composition. The bark used in the examples of the present invention is from the Sichuan, Guizhou and Yunnan provinces of China, and Northeast China.

2. Semen armeniacae amarum (Prunus seeds)

General Description

The dried, ripe seeds of Prunus armeniaca L. Var. ansu Maxim., Prunus sibirica L., Prunus mandshurica (Maxim) Koehne, or Prunus armeniaca L. are used in the composition. (family-Rosaceae) are used in the composition. The seed used in the examples of the present invention were collected in the Heilongjiang, Liaoning, Jilin, and Hebei provinces of China.

3. Radix stemonae (Stemona tubers)

General Description

The dried root tuber of Stemona sessilifolia (Miq) Miq., Stemona japonica (Bl.) Miq; or Stemona tuberosa Lour. (family-Stemonaceae) are used in the composition. The tubers used in the examples of the present invention were obtained in the Jiangsu, Anhui, Zhejiang, and Shandong provinces of China.

4. Semen coicis (Coix seeds)

General Description

The dried, ripe kernels of Coix lacryma-jobi L. Varma-yuen (Roman) Stapf (family-Grarnineae) are collected in the autumn and dried. The kernels are separated from the shells, tawny seed coats and any impurities. The kernels used in the examples of the present invention were collected in the Jiangsu, Fujian, Hebei and Liaoning provinces.

Identification

The starch grain turns brownish red when added to iodine test solution.

5. Rhizoma pinelliae (Pinellia tubers)

General Description

The dried stem tubers of Pinellia ternata (Thunb.) Breit. (family-Araceae) are used in the composition. The tubers used in the examples of the present invention were collected in the Sichan, Zhejiang, Anhui, and Jiangsu provinces of China.

6. **Fructus forsythiae** (Forsythia fruit)

General Description

The dried fruits of Forsythia suspensa (Thunb.) Vahl (family-Oleaceae) are used in the composition. The fruits used in the examples of the present invention were obtained from the Shanxi, Henan, Shaanxi and Shangdong provinces of China.

7. Radix stephaniae tetrandrae (Radix fangji) (Stephania roots)

General Description

The dried root of the plant Stephania tetrandra S. Moore (family-Menispermaceae) are used in the composition. The roots used in the examples of the present invention are from the Anhui, Zhejiang, and Jiangxi provinces of China.

8. Medulla tetrapanacis (Tetrapanax stems)

General Description

The dried stem medullas of Tetrapanax papyrferus (Hook.) K.Koch (family-Araliaceae) are used in the composition. The plants used in the examples of the present invention were harvested in the Guizhou, Yunnan, Guangxi and Sichuan provinces of China.

9. Herba siegesbeckiae (Siegesbeckia plants)

General Description

The aerial part of Siegesbeckia orientalis L., Siegesbeckia pubescens Makino, or Siegegsbeckia glabrescens Makino (family-Compositae). The plants used in the examples of the present invention were obtained from the Jiangsu, Hubei, and Hunan provinces of China.

10. Radix sophorae flavescentis (Radix Kuh-seng) (Sophora roots)

General Description

The dried root of Sophora flavescens Ait. (family-Leguminosae). The roots used in the examples of the present invention were obtained from the Shanxi, Henan, and Hebei provinces of China.

11. Radix et rhizoma tripterygii (Leigongteng) (Tripterygium roots and rhizomes)

General Description

Leigongteng consists of the dried root and rhizome of Tripterygium wilfordii Hook. f. (family: Celastraceae). The roots are cylindrical, twisted, often connected to the aerial stems, 60 cm or more in length, and varying in thickness with diameters of approximately 0.5 cm-3 cm. The epidermis is rough, khaki or orange/yellow in color, with irregular longitudinal microgrooves and transverse tissues. The cortex is easy to peel off but it is difficult to break. The fracture surface displays a brownish violet or brown phloem, a yellowish white or light brown xylem, and is densely covered by pin holes. The rhizome is thick and strong, with a rough epidermis, often greyish brown in color. Leigongteng is slightly aromatic in odor, bitter and acrid in taste. Leigongteng has strong toxicity and care must be taken in the administration so as to prevent an overdose. The plants utilized in the examples of the present invention were obtained from the Zhejiang, Fujian, and Hubei provinces of China.

Processing

The plant is gathered in the spring and autumn, cleaned, steeped in water for 4 to 6 hours, removed, and cut into thick slices, and dried out.

Identification

Test Number 1.

50 ml of ethyl alcohol are added to 5 g of powdered leigongteng, the resultant mixture is refluxed on a water bath for one hour and filtered, the filtrate is evaporated to dryness, 5 ml HCL (0.1 mol/L) is added to dissolve the dried residue, and the resultant mixture is filtered. The filtrate is halved into two test tubes. To one tube are added 2 drops of potassium bismuth iodide TS and a yellow precipitate is produced. To the other tube are added 2 drops of potassium mercuric iodide TS and a white precipitate is produced. Next, 5 ml ethyl acetate are added to dissolve the acid-water-insoluble portion, the resultant mixture is filtered, 2 drops of 2% TS of 3.5-dinitrobenzoic acid and 2 drops of 5% TS of sodium hydroxide are added to the filtrate and a purplish red color appears.

Test Number 2.

100 ml anhydrous ethyl alcohol are added to 20 g of powdered Leigongteng, a reflux extraction is conducted for 1 hour, the resultant mixture is filtered, and the filtrate is evaporated to dryness. 3 ml ethyl acetate are added to dissolve the residue, then 3 g of neutral aluminum oxide are added, the resultant mixture is agitated thoroughly, and the solvent is allowed to volatilize out of the mixture. Next, the mixture is packed into a column of neutral aluminum oxide (internal diameter 1.5 cm dry packing with 11 g of neutral AL2O3) and eluted with 100 ml of chloroform. All of the chloroform is recovered from the eluate. 0.5 ml anhydrous ethyl alcohol is added to the residue to make a sample solution. Separately, triptolide (reference substance) is used to make a standard solution (0.5 mg/ml). According to the thin layer chromatography (see page 57 of the Appendices of Chinese Pharmacopoeia, Vol. One, 1990), 10 mu l of the sample solution and 5 mu l of the standard solution are separately added to same silica gel G plate, developed with a mixture of chloroform and ethyl ether (2:1), removed, and dried by airing; the plate is first sprayed with 2% alcoholic solution of 3.5-dinitrobenzoic acid, and then with 8% alcoholic solution of potassium hydroxide. The chromatogram produced by the sample solution must show the same purplish red spots as that shown by the standard solution in the corresponding areas.

Storage

Store in dry, airy, mold and moth proof location.

12. Radix glycyrrhizae (Glycyrrhiza roots and rhizomes)

General Description

The dried root and rhizome of Glycyrrhiza uralensis Fisch., G. inflata Bat., or G. glabra L. (family-Leguminosae) are used in the composition. The roots and rhizomes used in the examples of the present invention are from the Inner Mongolia Autonomous Region and the Gansu, Shaanxi, Shanxi, and Olnghai provinces of China.

D. Proportion of Components in the Composition

The exact proportion of the Chinese herbs, pulvis talcum and excrementum bombycis in the composition will depend on the concentration of the active ingredients found naturally in each component. Using the guidance provided herein and a basic knowledge of drug preparation and pharmacology, one skilled in the art could easily adjust the proportions of the separate

components of the composition so as to obtain a composition which has the therapeutic effects discussed and shown in the examples herein. The following discussion regarding the proportions of ingredients in the composition are provided as examples only and in no way limit the scope of the present invention from including any novel combination of the disclosed herbal and non-herbal components which have the intended effect of relieving the symptoms of pain, fever and inflammation, as discussed herein.

Composition Example 1. Based on the Percentages of the Herbal and Non-herbal Components.

* Ingredient	Percentage of Ingredient in the Composition
Pulvis talci	1-10%
Excrementum bombycis	5-15%
Cortex phellodendri	5-15%
Semen armeniacae amarum	5-15%
Radix stemonae	1-10%
Semen coicis	1-10%
Rhizoma pinelliae	5-15%
Fructus forsythiae	5-15%
Radix stephaniae tetrandrae	5-15%
Medulla tetrapanacis	5-15%
Herba siegesbeckiae	1-10%
Radix sophorae flavescentis	5-15%
Radix et rhizoma tripterygii	1-15%
Radix glycyrrhizae	5-15%

Composition Example 2. Based on the Weight Ranges of Herbal and Non-herbal Components.

* Ingredient	Weight Range of Ingredient in the Composition
Pulvis talci	100-120 g
Excrementum bombycis	80-100 g
Cortex phellodendri	80-100 g
Semen armeniacae amarum	80-100 g
Radix stemonae	80-100 g
Semen coicis	170-190 g
Rhizoma pinelliae	100-120 g
Fructus forsythiae	170-190 g
Radix stephaniae tetrandrae	80-100 g
Medulla tetrapanacis	50-70 g
Herba siegesbeckiae	170-190 g
Radix sophorae flavescentis	80-100 g
Radix et rhizoma tripterygii	50-70 g
Radix glycyrrhizae	170-190 g

Composition Example 3. Based on the Exact Weight of Herbal and Non-herbal Components.

* Ingredient	Weight of Ingredient in the Composition
Pulvis talci	108 g
Excrementuin bombycis	90 g
Cortex phellodendri	90 g
Semen armeniacae amarum	90 g
Radix stemonae	90 g
Semen coicis	180 g
Rhizoma pinelliae (prepared)	108 g
Fructus forsythiae	180 g
Radix stephaniae tetrandrae	90 g
Medulla tetrapanacis	60 g
Herba siegesbeckiae	180 g
Radix sophorae flavescentis	90 g
Radix et rhizoma tripterygii	60 g
Radix glycyrrhizae	180 g

III. PREPARATION OF THE COMPOSITION

A. Preparation Procedure.

The composition of the present invention is made from twelve pure natural Chinese herbs, talc and silkworm excrement. The Chinese herbs are individually washed, dried and ground into fine powder, then extracted, for example, with medical ion exchange water and alcohol separately, and then mixed together. The resultant mixture is dried, smashed, screened, and then mixed with the talc and silkworm excrement. The final composition can be incorporated into any convenient mode of administration, with oral capsule intake being the preferred method of administration. Exemplified capsules each contain 0.4 g of the composition.

More specifically, the herb components radix stephaniae tetrandrae and radix et rhizoma tripterygii are ground into fine powder. The other ten herbs are decocted (i.e., boiled-down) with water twice: the first decoction uses water which weighs ten times as much as the total weight of the eleven herbs while the second decoction uses water which weighs eight times as much as the total herbal weight. The time for each decoction is two hours. The material resulting from the decoctions is then mixed and filtered so as to concentrate the filtrate to a heavy paste with a relative density of 1.30-1.35 (at 60-65o C.). Next, the powders of the pulvis talcum, excrementum bombycis, and the other two herbs are added to the heavy paste and the resultant product is mixed, dried, pulverized, sieved and mixed again until homogeneous. The resulting brown or greyish-brown powder, which is slightly bitter and sweet in taste, can be placed into capsules for oral administration to patients.

The primary active ingredients in the composition include tetrandrine, sophocarpidine, hydrochloric phellodendrine and saponin.

B. Quality Standards.

The quality standards for each of the herbs except radix et rhizoma tripterygii (leigongteng) are set forth in the Chinese Pharmacopeia (1990). The newly-devised assay test for radix et

rhizoma tripterygii is provided in Assay Test Number 5.

1. Assay Test Number 1.

Add 25 ml of 70% ethyl alcohol to 2 g of the composition,, treat with ultrasonic irradiation for 1 hour, and then let stand for 12 hours. Pipet 10 ml of the supernatant liquid into an evaporating dish, evaporate to near dryness on a waterbath. Transfer the residue into a separating funnel with 20 ml of water, add 1 ml of concentrated ammonium TS, shake well, and then extract with chloroform for three times using 15 ml of chloroform each time, combine the extracts and recover the solvent chloroform. Add 2 ml ethyl alcohol to dissolve the residue to make up the sample or test solution.

Separately, use 0.5 g of radix stephaniae tetrandrae and 0.5 g of radix sophorae flavescentis to prepare two reference solutions by the same procedure.

In addition, produce two more reference samples by separately adding tetrandrine and matrine to ethyl alcohol (1 ml:1 mg).

According to the Chromatography Procedure on page 57 of the Appendices of Chinese Pharmacopoei (Volume One, 1990 edition), pipet 10 mu L of the sample solution, and separately pipet 5 mu l of the four reference solutions to the same plate of silica gel G. Use a mixture of petroleum ether (30-60o C.), ethyl acetate, and diethylamine (7:2:1) as developer to develop the chromatogram. Remove the plate, air dry, and spray with a dilute solution of potassium bismuth iodide. The chromatogram produced by the sample solution must show the same orange-yellow spots as that displayed by each reference solution in their corresponding areas.

2. Assay Test Number 2.

Add 20 ml of ammonium solution (1 mol/L) to 2 g of the powder, stir well, macerate for 10 minutes, and filter. Add 0.5 ml of concentrated sulfuric acid to the filtrate, agitate well, and filter. Add 2 ml of an alcoholic solution of ammonium (1 mol/L) to the residue to dissolve it. The resulting solution is the sample or test solution.

Separately, use 1 g of the reference "licorice" to make up a reference solution, using the same procedure as set forth in Assay Test Number 1 (see above).

Also, use "ammonium glycyrrhizinate" to make another reference solution by adding to it to ethyl alcohol (1 ml:1 mg).

According to the Chromatography Procedure on page 57 of the Appendices of Chinese Pharmacopoeia (Volume One, 1990 edition), pipet 10 mu l of the sample solution, and separately pipet 4 mu l of the two reference solutions and apply them to the same plate of silica gel GF254. Use a mixture of n-butanol, glacial acetic acid, and water (4:1:2) as a developer to develop the chromatogram. Remove the plate, air dry, and observe under an ultraviolet lamp (254 nm). The chromatogram produced by the sample solution must have the same quenching fluorescent spots as that of each reference solution in their corresponding areas.

3. Assay Test Number 3.

Add 10 ml of ethyl alcohol to 1 g of the composition and store overnight at room temperature. Filter the solution and evaporate the filtrate to dryness. Dissolve the residue with 1 ml ethyl alcohol to produce the sample or test solution.

Separately, use radix phellodendri to make a reference solution (Ref Sol. No. 1) by the same

procedure as set forth in Assay Test Number 1 (see above).

Next, use berberine hydrochloride to make a second reference solution (Ref Sol. No. 2) by adding ethyl alcohol (1 ml:0.5 mg).

According to the Chromatography Procedure on page 57 of the Appendices of Chinese Pharmacopoeia (Volume One, 1990 edition), pipet 15 mu l of sample solution, 3 mu l of Ref. Sol. No. 1, and 1 mu l of Ref. Sol. No. 2 to the same plate of silica gel G. Use a mixture of ethyl acetate, n-butanol, formic acid, and water as a developer (10:1:1:1) to develop the chromatogram. Remove the plate, air dry, and observe it under an ultraviolet lamp (365 nm). The chromatogram produced by the sample solution must show the same yellow fluorescent spots as that shown by each of the reference solutions in their corresponding areas.

4. Assay Test Number 4.

Place 65 g of the composition into a Soxhlet apparatus, add petroleum ether (30-60o C.) Q.S., run a hot reflux extraction for 3 hours, discard the solvent petroleum ether, let the remaining solvent volatilize away from the remaining product, and put the resultant product into a reflux extractor with chloroform and extract for an additional 3 hours. Next, recover the solvent chloroform from the extract, add 3 ml of ethyl acetate to the residue to dissolve it, and quantitatively transfer it into an evaporating dish. Add 3 g of neutral aluminum oxide, mix well, and volatilize the ethyl acetate. Next, place the resultant mixture into a column of neutral aluminum oxide (internal diameter 1.5 cm with 11 g of neutral Al2O3 packed by a dry process), elute with a mixture of chloroform and anhydrous ethyl alcohol (9:1), collect the eluate, and recover all the solvent from the eluate. Add 0.5 ml of anhydrous ethyl alcohol to dissolve the residue to produce the sample or test solution.

Separately, use radix et rhizoma tripterygii to make a first reference solution (Ref Sol. No. 1) by the same procedure as set forth in Assay Test Number 1 (see above).

Separately, dissolve triptolide in anhydrous ethyl alcohol to make a second reference solution (Ref. Sol. No. 2) (1 mg:0.5 mg).

According to the Chromatography Procedure on page 57 of the Appendices of Chinese Pharmacopoeia (Volume One, 1990 edition), pipet 10 mu l of the sample solution and 3 mu l of each of the reference solutions to the same plate of silica gel G, with a mixture of chloroform and ethyl ether (2:1) as developer to develop the chromatogram. Remove, air dry, spray with a 2% alcoholic solution of 3,5-dinitrobenzoid acid, and then spray with an 8% alcoholic solution of potassium hydroxide. Cover the chromatoplate with a glass pane and fix by adhesive tape on every side. According to the thin-layer scanning on page 57 of the Appendices of Chinese Pharmacopoeia (Volume One, 1990 edition), scan the chromatoplate at the wave length of lambda [S] = 535nm and lambda [R] = 700 nm. Separately measure the quantities and absorbencies of the sample and the reference, and make the appropriate calculations.

Triptolide (C19H24O6) is the active component of leigongteng and can be quite toxic at higher concentrations. However, the amount of triptolide in raw leigongteng is very low, ranging from approximately 3.0-15.6 mu g per gram of fresh weight. Using the methods of the present invention, the amount of triptolide contained in each 0.4 g of the composition (i.e., the usual amount in one capsule for oral administration) will be 0.2-1.0 mu g per capsule. This low amount of triptolide in the compositions of the present invention is non-toxic and does not cause detrimental side effects as demonstrated by the animal tests and clinical studies reported herein.

5. Assay Test Number 5.

Use the sample solution and the two reference solutions prepared in Assay Test Number 5 (see above) for this assay.

According to the Chromatography Procedure on page 57 of the Appendices of Chinese Pharmacopoeia (Volume One, 1990 edition), pipet 10 mu l of the sample solution and 5 mu l of each of the reference solutions to the same plate of silica get G. Use a mixture of chloroform and ethyl ether as developer (2:1) to develop the chromatogram. Remove the plate and air dry. First spray the plate with a 2% alcoholic solution of 3,5-dinitrobenzoic acid and then spray it with an 8% alcoholic solution of potassium hydroxide. The chromatogram produced by the sample solution must show the same purple spots as that shown by the reference solutions in their corresponding areas.

6. Assay Test Number 6.

Place 5 g of the composition into a stoppered conical flask, add 50 ml of 70% ethyl alcohol, stopper, treat with ultrasonic irradiation for 1 hour, and let stand for 12 hours. Pipet 20 ml of the supernatant liquid into an evaporating dish, and evaporate using a waterbath until about 3 ml of the solutions is left. Transfer the resultant solution into a separating funnel with 50 ml water, add 2 ml of concentrated ammonium TS, shake well and then extract with chloroform five times, using 30 ml of chloroform for each extraction. Combine the dehydrated chloroform liquid with anhydrous sodium sulfate, recover all the solvent chloroform, dissolve the residue in anhydrous ethyl alcohol and quantitatively transferred into a 5-ml volumetric flask. Next, dilute to 5 ml with anhydrous ethyl alcohol, and shake well. The resultant solution is the sample or test solution.

To prepare a reference solution, add tetrandrine (the reference substance) to anhydrous ethyl alcohol (1 mg:0.5 mg).

According to the Chromatography Procedure on page 57 of the Appendices of Chinese Pharmacopoeia (Volume One, 1990 and edition), pipet 5 mu l and 8 mu l of the sample solution and 3 mu l and 5 mu l of the reference solution in a cross pattern on the same plate of silica gel G. Use a mixture of petroleum ether (30-60o C.), ethyl acetate, and diethylamine (7:2:1) as a developer to develop the chromatogram. Air dry, spray with a dilute solution of potassium bismuth iodide until wet and the spots appear clearly. Cover the chromatoplate with a glass pane and fix by adhesive tape on every side. According to the thin-layer scanning on page 57 of the Appendices of Chinese Pharmacopoeia (Volume One, 1990 edition), scan the chromatogram at the wavelengths lambda [S] = 500 nm and lambda [R] = 700 nm. Measure the quantities of absorbencies from the sample and the reference and make appropriate calculations.

The amount of radix stephaniae tetrandrae contained in each 0.4 g of the composition (i.e., the usual amount in one capsule used for oral administration) should not be less than 250 mu g (based on tetrandrine (C38H42N2O6) being the principle component).

IV. STORAGE STABILITY OF THE COMPOSITION

Storage Stability Study. Three samples were obtained each from a different batch of the composition and placed in plastic bottles which were sealed tightly and left standing at room temperature for one year. The average room temperature at four different sampling times are shown in Table 1.

TABLE 1

Average room temperatures for four sampling times.

Date	Temperature	Relative Humidity
March, 1994	16.4o C.	79.2%
June, 1994	29.2o C.	76.5%
December, 1994	10.3o C.	62.6%
March, 1995	17.0o C.	79.3%

The various tests and the test results for each of the three batches are shown in Tables 2, 3 and 4.

TABLE 2

Storability tests for Batch No. 940301. Mfg date: March 12, 1994.

Standing time (Inspection date)	*	*	*	
Results	0 month	3 months	9 months	12 months
Items	(Mar 94)	(June 94)	(Dec 94)	(Mar 95)
Description	greyish-brown	as before	as before	as before
Identification				
1. Microscopic Identification	showing the feature stipulated	ditto	ditto	ditto
2. Chromatographs of Fangji root & Kuh-seng root	positive reaction	ditto	ditto	ditto
3. Chromatograph of Licorice	positive reaction	ditto	ditto	ditto
4. Chromatograph of Cortex phellodendri	positive reaction	ditto	ditto	ditto
5. Chromatograph of Leigongteng	positive reaction	ditto	ditto	ditto
Determination				
Water content %	4.3	4.3	4.6	5.0
Dissolution time (m)	15	15	16	16
Triptolide (mu g/cap)	0.45	0.43	0.41	0.41
Assay:				
Tetrandrine (mu g/cap)	300	298	295	294
Hygienic test				
Bacteria	< 10	< 10	< 10	< 10
Fungi	< 10	< 10	< 10	< 10
Pathogenic bacteria	undetected	ditto	ditto	difto
Live acarids	undetected	ditto	ditto	ditto

TABLE 3

Storability tests for Batch No. 940302. Mfg date: March 15, 1994.

Standing time (Inspection date)	*	*	*	
Results	0 month	3 months	9 months	12 months
Items	(Mar 94)	(June 94)	(Dec 94)	(Mar 95)
Description	greyish-brown	as before	as before	as before
Identification				
1. Microscopic Identification	showing the feature stipulated	ditto	ditto	ditto
2. Chromatographs of Fangji root & Kuh-seng root	positive reaction	ditto	ditto	difto
3. Chromatograph of Licorice	positive reaction	ditto	ditto	ditto
4. Chromatograph of Cortex phellodendri	positive reaction	ditto	ditto	ditto
5. Chromatograph of Leigongteng	positive reaction	ditto	ditto	ditto
Determination				
Water content %	5.8	5.8	6.0	6.1
Dissolution time (m)	18	19	19	20
Triptolide (mu g/cap)	0.58	0.58	0.51	0.50
Assay:				
Tetrandrine (mu g/cap)	302	302	302	301
Hygienic test				
Bacteria	50	50	50	100
Fungi	< 10	< 10	< 10	< 10
Pathogenic bacteria	undetected	ditto	ditto	ditto
Live acarids	undetected	ditto	ditto	dittd

TABLE 4

Storability tests for Batch No. 940303. Mfg date: March 9, 1994.

Standing time (Inspection date)	*	*	*	
Results	0 month	3 months	9 months	12 months
Items	(Mar 94)	(June 94)	(Dec 94)	(Mar 95)
Description	greyish-brown	as before	as before	as before
Identification				

1. Microscopic Identification	showing the feature stipulated	ditto	ditto	ditto
2. Chromatographs of Fangji root & Kuh-seng root	positive reaction	ditto	ditto	ditto
3. Chromatograph of Licorice	positive reaction	ditto	ditto	ditto
4. Chromatograph of Cortex phellodendri	positive reaction	ditto	ditto	ditto
5. Chromatograph of Leigongteng	positive reaction	ditto	ditto	ditto
Determination				
Water content %	4.6	4.8	4.9	4.9
Dissolution time (m)	16	16	17	17
Triptolide (mu g/cap)				
Assay:	0.53	0.51	0.48	0.45
Tetrandrine (mu g/cap)	301	301	301	302
Hygienic test				
Bacteria	< 10	< 10	< 10	100
Fungi	< 10	< 10	< 10	< 10
Pathogenic bacteria	undetected	ditto	ditto	ditto
Live acarids	undetected	ditto	ditto	ditto

As demonstrated by the results presented above, the composition of the present invention is stable when stored for one year at room temperature in a closed plastic bottle. Every one of the tests conducted on the three batches of stored composition show no loss of quality when compared to freshly-prepared compositions.

V. PHARMACEUTICAL FORMULATIONS

The herbal components and non-herbal components of this invention can be used in the form of a medicinal preparation, for example, in solid, semi-solid or liquid form which contains the composition of the present invention, as an active ingredient, in admixture with an organic or inorganic carrier or excipient suitable for external, enteral or parenteral applications. The active ingredient may be compounded, for example, with the usual non-toxic pharmaceutically acceptable carriers for tablets, pellets, capsules, suppositories, solutions, emulsions, suspensions, and any other form suitable for use. Formulations of the present invention encompass those which include the exemplified carrier talc, as well as carriers other than talc such as water, glucose, lactose, gum acacia, gelatin, mannitol, starch paste, magnesium trisilicate, corn starch, keratin, colloidal silica, potato starch, urea and other carriers suitable for use in manufacturing preparations, in solid, semisolid or liquid form and in addition auxiliary, stabilizing, thickening and coloring agents and perfumes may be used.

For preparing solid compositions such as tablets or capsules, the principal active ingredients are mixed with a pharmaceutical carrier (e.g., conventional tableting ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium

phosphate or gums) and other pharmaceutical diluents (e.g., water) to form a solid preformulation composition containing a substantially homogeneous mixture of a composition of the present invention, or a non-toxic pharmaceutically acceptable salt thereof. When referring to the preformulation compositions as substantially homogenous, it is meant that the active ingredients are dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation composition is then subdivided into unit dosage forms of the type describe d above containing 0.4 m g of the composition of the present invention, preferably in capsules. The tablets or pills of the novel composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol and cellulose acetate.

The liquid forms, in which the novel composition of the present invention may be incorporated for administration orally or by injection, include aqueous solution, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil as well as elixirs and similar pharmaceutical vehicles. Suitable dispersing or suspending agents for aqueous suspensions include synthetic natural gums, such as tragacanth, acacia, alginate, dextran, sodium carboxymethyl cellulose, methylcellulose, polyvinylpyrrolidone or gelatin.

Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for reconstitution with water or other suitable vehicles before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, methyl cellulose or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters or ethyl alcohol); preservatives (e.g., methyl or propyl p-hydroxybenzoates or sorbic acid); and artificial or natural colors and/or sweeteners.

For buccal administration, the composition may take the form of tablets or lozenges formulated in conventional manners.

The active compounds may be formulated for parenteral administration by injection, which includes using conventional catheterization techniques or infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampules, or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulating agents such as suspending, stabilizing, and/or dispersing agents. Alternatively, the active ingredients may be in powder form for reconstitution with a suitable vehicle, e.g., sterile pyrogert-free water, before use.

DETDESC:

VI. EXAMPLES

A. Non-Human Animal Trials

Pharmacologic research shows that the composition of the present invention has an antiinflammatory effect on metatarsal enlargements as well as preventing or alleviating primary and secondary pathological conditions from arthritis of hamsters caused by

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administering a substance which causes inflammation. The composition of the present invention also provides dramatic antiinflammatory results due to carragheenin-induced edema in mice and albumin-induced edematous footpad in rats. In addition, the composition promotes blood flow and removes circulatory obstruction as shown by tests on the permeability of capillaries and modeling of granulomatosis in hamsters. Fever caused by a saccharomyces infection can be reduced for over 4 hours by taking the composition of the present invention. The composition of the present invention can also reduce writhing reaction created by chemical stimulus such as by acetic acid and also provide a painkilling effect.

General Experimental Method

The compositions used in these experiments were prepared by the Pharmaceutical Preparation Room of Taiyuan Rheumatoid Arthritis Hospital using the procedures outlined above. Distilled water was added to prepare a suspension of the density required for the tests.

Aspirin (99.6%) was manufactured by Nanjing Pharmacy. A 10% of gum acacia solution was added to prepare a suspension of the density required for the test.

Distilled water was used for the control groups.

The experimental mice and Wistar Rats were bought from the Animal Experiment Centre of Shanxi Medical College.

Animal Study 1

Inhibitory Action of the Composition on Primary Arthritis Caused by Freund's Complete Adjuvant

Male rats were randomly divided into four groups of ten rats each, with the total weight of each group being 172.5 +/- 8.0 g. The perimeters of the rat hind paws were measured. Each group of rats was fed either water (control), aspirin (0.15 g/kg of body weight), or the composition of the present invention (either 1.5 g or 3.0 g/kg of body weight) by means of gastrogavage, once a day for 3 days. On the third day, 1 hour after the gastrogavage, 0.1 ml of Freund's complete adjuvant was intradermally injected into each rat's right hind paw to induce inflammation. The perimeters of the left and right hind paws were measured 2, 4, 6, 24, 48 and 72 hours after inflammation. As shown in Table 1, the composition of the present invention has an inhibitory action on the inflammation of primary arthritis caused by administration of Freund's complete adjuvanticity.

TABLE 1

Inhibitory effect of the inflammation of primary arthritis caused by injection of Freund's complete adjuvant.

Dose	* No. of	Swelling Measurement (x mm +/- SD)

Treatment	g/kg	Rats	2h	4h	6h	24h	48h	72h
Control	*	10	6.4 +/- 1.17	9.20 +/- 0.92	6.50 +/- 1.43	6.10 +/- 0.88	6.00 +/- 1.49	5.50 +/- 2.37
Compo-	3	10	4.40 +/- 0.96	5.80 +/- 1.03	5.40 +/- 0.97	4.70 +/- 1.34	5.40 +/- 1.35	5.80 +/- 2.75
sition	*	*	**	**	*	**		
Compo-	1.5	10	4.60 +/- 1.43	6.80 +/- 1.62	5.50 +/- 1.18	4.80 +/- 1.03	5.90 +/- 1.79	6.70 +/- 1.95
sition	*	*	**	**	*	**		
Aspirin	0.15	10	2.40 +/- 1.08	4.40 +/- 1.57	5.30 +/- 1.34	3.20 +/- 092	5.01 +/- 1.92	5.80 +/- 1.75
	*	*	**	**	*	**		

nTest. Compared with the Control. *P < 0.05. **P < 0.01 -

Animal Study 2

Inhibitory Action of the Composition on Primary Arthritis Caused by Freund's Complete Adjuvant

Male rats were randomly divided into four groups often rats each, with the total weight of each group being 172.5 +/- 8.0 g. The perimeters of the left and right rat hind paws were measured. 0.1 ml of Freund's complete adjuvant was intradermally injected into each rat's right hind paw to induce inflammation. One week after injection of Freund's complete adjuvant, each group of rats was fed either water (control), aspirin (0.15 g/kg of body weight), or the composition of the present invention (either 1.5 g or 3.0 g/kg of body weight) by means of gastrogavage. The hind paws were observed and measured 1, 4 and 7 days after receiving treatments of either water, aspirin or the composition of the present invention. Results of the treatments were determined by measuring the degree of swelling of the injected versus non-injected hind paws, changes in body weight, the number of tubercles, and an overall visual score of the difference between the injected and non-injected paws. The visual score included observations on erythema of the ears and the swelling of the hind paws, anklejoints, and paw joints. The results are provided in Table 2. Compared with the control group, the swelling of the rats' paws after injection of the Freunds' complete adjuvant injection was significantly reduced for rats receiving either composition and compared favorably with the results of the aspirin treatment. The number of tubercles and the visual scores were also significantly lower for the rats treated with the composition as compared to the control group. No obvious changes were noted in rat body weight over the treatment regime.

TABLE 2

Inhibitory effect on the inflammation of primary arthritis caused by injection of Freund's complete adjuvant.

	No.	Swelling	Changes in Body Weight	No. of

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Treatment	Dose g/kg	of Rats	(x mm +/- SD)	After 1 Day	After 4 Days	After 7 Days	Tub-ercles	Visual Score
Control	*	10	1.3 +/- 0.67	165.3 +/- 5.0	182.0 +/- 11.2	200.4 +/- 17.2	8	22
Composition	3 *	10 *	0.3 +/- 0.94 **	174.8 +/- 8.5	190.7 +/- 14.2	210.3 +/- 10.4	0	0
Composition	1.5 *	10 *	0.4 +/- 0.87 *	175.3 +/- 11.6	183.3 +/- 15.5	209.0 +/- 18.4	1	1
Aspirin	0.15 *	10 *	0.0 +/- 0.57 **	174.5 +/- 6.6	188.0 +/- 5.0	215.3 +/- 13.3	1	1

nTest: Compared with the control. P < 0.05, **P < 0.01 -

Animal Study 3

Anti-inflammatory Function of the Composition on the Carragheenin Induced Edematous Footpad in Mice

This test was conducted according to the procedure established by Xu-Shu-Yun, Methodology of the Pharmacologic Experiments, The People's Public Health Publishing House, 717 (1991). Male mice were randomly assigned to one of four groups of ten mice each, each group weighing 22.5 +/- 2.1 g. Each group of mice was fed either water (control), aspirin (0.15 g/kg of body weight), or the composition of the present invention (either 1.5 g or 3.0 g/kg of body weight) by means of gastrogavage, once a day for 8 days. On the eighth day, 1 hour after the gastrogavage, 0.1 ml of a 1.0% carrageenin solution was hypodermically injected into the right ankle joint. Using a volumometer, the volumes of the paw and ankle joints were measured 0.5, 1, 2, 3 and 4 hours after inflammation and the values were used to calculate a swelling value. As shown in Table 3, the composition of the present invention had a significant inhibitory effect on swelling and this positive effect lasted at least 4 hrs.

TABLE 3

Inhibitory effect of the composition on carrageen-induced edematous footpad in mice.

Treatment	* Dose g/kg	No. of Mice	Degree of Swelling of Hind Paw for a Given Time After Injection (x ml +/- SD) 0.5 hr	1 hr	2 hr	3 hr	4 hr
Control	*	10	0.10 +/- 0.03	0.13 +/- 004	0.08 +/- 0.03	0.06 +/- 0.05	0.04 +/- 0.03
Composition	3 *	10 *	0.08 +/- 0.02 *	0.11 +/- 0.03 *	0.02 +/- 0.02 **	0.02 +/- 0.03 *$H**	0.01 +/- 0.02
Composition	1.5	10	0.09 +/- 0.02	0.13 +/- 0.03	0.05 +/- 0.03	0.03 +/- 0.03	0.01 +/- 0.01

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			*	*	*		*		*	**
Aspirin	0.15	10	0.08 +/- 0.03		0.12 +/- 0.03		0.03 +/- 0.02		0.01 +/- 0.01	0.01 +/- 0.0
			*	*	*		*		**$H**	

nTest: Compared with the control *P < 0.05, **P < 0.01 -

Animal Study 4

Inhibitory Action of the Composition on Albumin-induced Edema of Rat hind Paw

This test was conducted according to the procedure established by Chen Qi, The People's Public Health House, 356 (1993). Rats were randomly divided into 4 groups of ten rats each, each group weighing 160.5 +/- 16.2 g. Each group of rats was fed either water (control), aspirin (0.15 g/kg of bodyweight), or the composition of the present invention (either 1.5 g or 3.0 g/kg of body weight) by means of gastrogavage, once a day for 3 days. On the third day, 1 hour after the gastrogavage, 0.1 ml of a 10.0% fresh egg white solution (diluted with normal saline) was hypodermically injected into the rat hind paw. The perimeters of the hind ankle joints were measured at different times in order to calculate degree of swelling. As shown in Table 4, the composition of the present invention had a significant effect on the degree of swelling when compared to the controls.

TABLE 4

Inhibitory effect on the albumin-induced edematous footpad.

			Degree of Swelling of Hind Paw for a Given Time After Injection (x ml +/- SD)					
Treatment	* Dose g/kg	No. of Mice	0.5 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Control	*	10	9.1 +/- 0.9	8.2 +/- 0.9	7.0 +/- 0.9	5.3 +/- 1.2	4.0 +/- 0.7	4.5 +/- 0.8
Composition	3	10	6.8 +/- 1.1	4.7 +/- 2.2	3.6 +/- 1.7	3.0 +/- 1.9	2.3 +/- 1.4	2.5 +/- 1.1
	*	*	**	**	**	**	**	**
Composition	1.5	10	8.0 +/- 0.7	6.0 +/- 1.3	5.6 +/- 1.8	3.4 +/- 1.1	3.8 +/- 1.0	4.1 +/- 1.2
	*	*	**	**	*$H**			
Aspirin	0.15	10	7.0 +/- 0.8	5.1 +/- 1.2	4.5 +/- 1.5	3.4 +/- 1.3	3.2 +/- 1.0	3.2 +/- 1.4
	*	*	**	**	**	**	*	*

nTest: Compared with the control. *P.0.05, **P < 0.01. -

Animal Study 5

Inhibitory Effect of the Composition on Mice Capillary Permeability Induced by Glacial Acetic Acid

This test was conducted according to the procedure established by Chen Qi, The People's Public Health House, 303 (1993). Mice were randomly assigned to one of 4 groups, each group having a total weight of 21.8 +/- 1.9 g and consisting of 5 male and 5 female mice each. Each group of mice was fed either water (control), aspirin (0.15 g/kg of body weight), or the composition of the present invention (either 1.5 g or 3.0 g/kg of body weight) by means of gastrogavage. One hour after the gastrogavage, a solution consisting of 0.5% Evans blue and (I.P.) 0.7% glacial acetic acid 0.1 ml/10 g (body weight) was hypodermically injected into the mice. I.P. densitometry values were collected 20 minutes after the injection. The results are presented in Table 5. Compared with the control group, the composition of the present invention significantly inhibited increased capillary permeability.

TABLE 5

Inhibitory effect of the composition on increased capillary permeability induced by I.P. 0.7 acetic acid in mice.

* Treatment	Dose g/kg	No. of Mice	Evans blue (densitometry) (x +/- SD)
Control	*	10	0.479 +/- 0.14
Composition	3	10	0.215 +/- 0.08**
Composition	1.5	10	0.233 +/- 0.08**
Aspirin	0.15	10	0.231 +/- 0.07**

nTest: Compared with the control. **P < 0.01. -

Animal Study 6

Inhibitory Effect of the Composition on the Hyperplasia of Connective Tissues

This test was conducted according to the procedure established by the Medication Dept. of the Public Health Ministry (Collection of Guiding Principles on Western Medicine's Pre-Clinical Research, 271 (1993)). Rats were randomly assigned to one of 4 groups, each group consisting of 8 rats with a total weight 157.8 +/- 9.0 g. The rats in each group were anesthetized, tiny incisions were cut on the left and right groins, and 10 mg of sterilized cotton was placed inside each cut. Each group of rats was fed either water (control), aspirin (0.15 g/kg of body weight), or the composition of the present invention (either 1.5 g or 3.0 g/kg of body weight) by means of gastrogavage, once daily for 8 days. On the day following the last gastrogavage, the cotton and any granuloma deposits thereon were removed from the incisions, dried at a temperature of 60o C. for 12 hours, after which they were individually weighed. The weight of the granuloma deposits was calculated by subtracting the dry weight of the removed cotton from the original weight of the cotton before insertion (i.e., 10 mg). The resulting values were converted into the dry weight of granuloma per 100 g of rat weight. The Inhibitory Ratio is calculated as follows:

Inhibitory Ratio = ((control group average value - treatment group average)/(control group average)) x 100.

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As shown in Table 6, the composition of the present invention had a significant inhibitory action on the hyperplasia of connective tissues (granulomatosis). The result provides scientific evidence that the composition of the present invention is useful for the treatment of acute rheumatoid arthritis.

TABLE 6

Inhibitory effect on granulomatosis induced by Cotton Pallet Method in rats

Treatment	* Dose g/kg	* No. of Rats	Dry Weight of the granuloma (x-mg/100 +/- SD)	Inhibitory rate %
Control	*	8	12.3 +/- 1.3	
Composition	3	8	8.9 +/- 1.0**	27.64
Composition	1.5	8	10.0 +/- 1.9*	18.70
Aspirin	0.1	8	10.1 +/- 1.6**	17.89

nTest: Compared with the control. -

n*P < 0.05, **P < 0.01. -

Animal Study 7

Anti-pyretic Function of the Composition

This test was conducted according to the procedure established by Chen Qi, The People's Public Health House, 271 (1993).

The rats were randomly divided into 4 groups, each group weighing 210.0 +/- 10.9 g and consisting of 5 male and 5 female rats each. Rats were maintained in the test environmental conditions for 3 days during which their anal temperatures were recorded twice daily. On the fourth day the anal temperature was measured every half hour. Next, a 15% solution of fresh yeast suspension (2 ml/100 g) was injected into the back of each rat. Anal temperature was measured 4 hours later. The rats whose temperatures rose above 1o C. were divided into 4 groups, each group consisting of 10 rats. Each group of rats was fed either water (control), aspirin (0.15 g/kg of body weight), or the composition of the present invention (either 1.5 g or 3.0 g/kg of body weight) by means of gastrogavage. Anal temperatures were determined 1, 2, 3 and 4 hours after the gastrogavage. As shown in Table 7, the mice that received the composition had lower temperatures than the control mice, with many of the differences being statistically significant. This result demonstrates that the composition of the present invention has an anti-pyretic (i.e., fever reduction) activity in rats with fever caused by yeast injection.

TABLE 7

Anti-pyretic action of the composition on pyrexial rat caused by the injection of fresh yeast.

Treatment	Dose g/kg	No. of Rats	Normal Body Temp. (oC.)	Body Temp. 4 hrs after injection with yeast (oC.)	Changes in Body Temperature after injection with yeast (oC. x +/- SD) 1 hr	2 hr	3 hr	4 hr
Control	*	10	37.5 +/- 0.5	39.0 +/- 0.4	39.4 +/- 0.5	39.5 +/- 0.4	39.3 +/- 0.4	39.4 +/- 0.4
Composition	3	10	37.5 +/- 0.1	39.0 +/- 0.4	38.3 +/- 0.5 **	38.0 +/- 0.5 **	38.0 +/- 0.7 **	37.9 +/- 0.7 **
Composition	1.5	10	37.8 +/- 0.2	39.1 +/- 0.3	38.9 +/- 0.5 *	38.3 +/- 0.8 **	38.4 +/- 0.6 **	38.9 +/- 0.9
Aspirin	0.15	10	37.5 +/- 0.5	39.2 +/- 0.4	37.9 +/- 0.9 **	37.6 +/- 09 **	37.8 +/- 1.0 **	38.3 +/- 0.7 **

n Test: Compared with the control *P < 0, 05, **P < 0.01 -

Animal Study 8

Effect of the Composition on Writhing Activity

This test measures the analgesic action of the composition on writhing caused by injection of 0.5% acetic acid according to the procedure established by Chen Qi, Methodology of Chinese Herbs Pharmacologic Research, The People's Public Health Publishing House, 378 (1993).

The mice are randomly divided into 4 groups, each of which consists of 10 male and 10 female mice, with the total weight of each group being 20.9 +/- 1.1 g. Each group of mice was fed either water (control), aspirin (0.15 g/kg of body weight), or the composition of the present invention (either 1.5 g or 3.0 g/kg of body weight) by means of gastrogavage. One hour after the gastrogavage, a solution of (I.P.) 0.5% acetic acid (0.2 ml/20 g) was injected into the mice. The number of writhing reactions was counted during the 30 minutes immediately following the injection. As shown in Table 8, the composition of the present invention significantly reduced the writhing caused by injection of acetic acid into the mice.

TABLE 8

Analgesic action of composition on writhing reaction caused by injection of 0.5% of acetic acid

* Treatment	Dose g/kg	* Mice	Writhing Quantity (x +/- SD)	Analgesic Rate (%)
Control	*	20	66.5 +/- 23.4	
Composition	3	20	27.5 +/- 15.8**	58.65
Composition	1.5	20	31.5 +/- 17.6**	52.63
Aspirin	0.2	20	24.4 +/- 10.0**	63.31

nTest: Compared with the control **P < 0.01 -

B. Human Trials

Based on an ongoing series of pharmacological studies conducted by the China Academy of Traditional Chinese Medicine as well as an ongoing series of experimental studies with various herbal medicines throughout China, the composition of the present invention has been demonstrated to provide quick efficacy, a short treatment duration, no toxicity, and no adverse side effects. The results of the pharmacological study showed that the composition acts as an analgesic and a demulcent, relieves fever, reduces inflammation and promotes immuno-regulation. Significant efficacy has been achieved as a hormonal replacement for the treatment of rheumatoid arthritis, spondylarthritis ankylopoietica, systemic lupus erythematosus, erthema nodosum, scleroderma, Behcet's disease and Sjogren's syndrome. The composition is especially noted for controlling the effects of rheumatoid arthritis and for preventing joint deformity. Based on the observations of patients treated with the composition, including histopathological examinations on viscera, the composition caused no adverse effects to the hemogram or to liver/renal functions. In addition, no pathological problems were observed in any of the patients which were the result of administering the composition of the present invention. Considering clinical symptoms, signs, and lab indices, the composition has been demonstrated to markedly improve arthralgia and arthroncus and decrease inflammatory index. Based on the clinical studies, 97.2% of the patients had at least some relief of symptoms, while the overall curative rate was 38.52%. The dosage of the composition can be gradually reduced to a minimum maintenance dosage. Follow-up studies and clinical check-ups have shown that the post-curative effect is sustained and stable. To date, no cases of adverse reaction have been found.

The therapeutic composition of the present invention is normally administered using oral capsules each containing 0.4 g of the powder. Adults normally receive 3-5 capsules taken orally before meals three times daily. Based on their age and weight, children receive a lower dosage than adults.

Four typical cases are as follows:

Case Study 1. Patient 1-Age: 30; Sex: Male; Place of birth: Daxian, Sichuan; Occupation: peasant.

Condition before treatment: Overall swelling/painful joints for 18 years, both hands and elbows were deformed for 10 years, moved slowly with crutches.

Condition after treatment: After taking the composition for 30 days, the swelling and pain were completely relieved. Patient can now walk independently and take care of himself.

Case Study 2. Patient 2-Age: 29; Sex: Male; Place of birth: Huaiyuan, Anhui; Occupation: Worker

Condition before treatment: Painful hip/knee joints for 15 years, rigid and stiff waist for 10 years, limited activity for 3 years, kyphotic bending. Patient height was 174 cm.

Condition after treatment: After taking the composition for 21 days, the hip/knee joint pains was completely relieved. Sixty days later, patient had could move flexibly and his fully-extended height reached 176 cm.

Case Study 3. Patient 3-Age: 47; Sex: Male; Place of birth: Kowloon, Hong Kong; Occupation: Businessman

Condition before treatment: Spondylarthritis ankylopietica for 29 years. Despite an artificial joint replacement of his left hip and synovectomy of his right knee joint, his condition kept worsening. He moved with a wheelchair and drank via a hose, and his neck was stiff.

Condition after treatment: After taking the composition for 60 days, he could walk independently. Eight months later, he was able to hike and climb mountains.

Case Study 4. Patient 4-Age: 30; Sex: Female; Place of Birth: Taiyuan, Shanxi; Occupation: Unknown.

Condition before treatment: During her study in Japan, she had acute rheumatoid arthritis. Treatment of her conditions in Japan was not very effective. She return to China in a wheelchair.

Condition after treatment: After taking the composition for 20 days, she was well again and could walk up and down stairways. Sixty days later, she was able to walk normally.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

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CLAIMS: What is claimed:

[*1] 1. A composition comprising talc, silkworm excrement, the root of Stephania, kernels of Coix, rhizomes of Pinellia, seeds of Prunus, bark of Phellodendron, roots of Sophor, stem of Tetrapanax, root tubers of Stemona, roots and rhizomes of Glycyrrhiza, roots and rhizomes of Tripterygium, fruit of Forsythia and the above-ground parts of Siegesbeckia.

[*2] 2. The composition of claim 1 wherein the plants are Stephania tetrandra, Coix lachryma-jobi, Pinellia ternata, Prunus mandshurica, Phellodendron amurense, Sophora flavescens, Tetrapanax papyriferus, Stemona japonica, Glycyrrhiza uralensis, Tripterygium wilfordii, Forsythia suspensa and Siegesbeckia glabrescens.

[*3] 3. The composition of claim 1 wherein the silkworm excrement is obtained from Bombyx mori.

[*4] 4. The composition of claim 1 wherein the Prunus plants are selected from the group consisting of Prunus armeniaca, Prunus sibirica, and Prunus mandshurica.

[*5] 5. The composition of claim 1 wherein the Phellodendron plants are selected from the group consisting of Phellodendron amurense and Phellodendron chinense.

[*6] 6. The composition of claim 1 wherein the Stemona plants are selected from the group consisting of Stemona japonica, Stemona tuberosa and Stemona sessilifolia.

[*7] 7. The composition of claim 1 wherein the Siegesbeckia plants are selected from the group consisting of Siegesbeckia glabrescens, Siegesbeckia orientalis and Siegesbeckia pubescens.

[*8] 8. The composition of claim 1 wherein the Glycyrrhiza plants are selected from the group consisting of Glycyrrhiza uralensis, Glycyrrhiza inflata and Glycyrrhiza glabra.

[*9] 9. The composition of claim 1 wherein the composition is in a form selected from the group consisting of powder, capsule, tablet, liquid, and caplet.

[*10] 10. The composition of claim 9 wherein the composition is in the form of a capsule.

[*11] 11. A method for reducing inflammation and pain in a mammal comprising administering a therapeutically effective amount of the composition of claim 9.

[*12] 12. The method of claim 11 wherein the mammal is a human.

[*13] 13. A method for alleviating symptoms associated with rheumatism comprising administering a therapeutically effective amount of the composition of claim 1 to a mammal.

[*14] 14. The method of claim 13 wherein the rheumatism is rheumatoid arthritis.

[*15] 15. A method for alleviating symptoms associated with arthritis comprising administering a therapeutically effective amount of the composition of claim 1 to a mammal.

[*16] 16. The method of claim 15 wherein the arthritis is osteoarthritis.

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Tsai , et al. **November 23, 1999**

Compositions of matter useful in the treatment of viral infections derived from plant extracts

Abstract

Compositions derived from Chinese herbal medicines, medicinal plants and extracts thereof, are provided for the treatment of animals infected with viruses, especially with hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). More specifically, the compositions of the present invention are derived from various Chinese herbal medicines or medicinal plants which have a long history of human consumption. The compositions of the invention are obtained through specific techniques and have demonstrated outstanding efficacy for treating human HBV carriers and hepatitis C patients. Compositions according to the invention have also exhibited in vitro antiviral activities against murine leukemia virus (MuLV) and HIV. HIV is the virus known to cause acquired immunodeficiency syndrome (AIDS) in humans and AIDS presents special problems to the medical community which the present invention addresses. Preferred compositions contain the herbal ingredients AEGINETIAE HERBA, BLECHNI RHIZOMA, LESPEDEZAE HERBA, POLYGONI CUSPIDATI RHIZOMA, FORSYTHIAE FRUCTUS, and LIGUSTRI FRUCTUS, or contain the herbal ingredients AEGINETIAE HERBA, LONICERAE FLOS, PRUNELLAE SPICA, and LESPEDEZAE HERBA.

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Parent Case Text

RELATED APPLICATIONS

This application claims priority to a provisional application filed Jul. 9, 1996, Ser. No. 60/016,100 entitled: ANTI-VIRAL AGENTS; and to a provisional application filed Jul. 10, 1996, Ser. No. 60/021,467 entitled: ANTI-VIRAL AGENTS FROM CHINESE MEDICINAL HERBS.

Claims

We claim:

1. A composition of matter useful in the treatment of viral infections, said composition comprising:

a) AEGINETIAE HERBA, prepared from an aqueous extract of the whole plant of at least one plant selected from the group consisting of Aeginetia indica, Dichondra micrantha, Striga lutea and Dichondra repens;

b) BLECHNI RHIZOMA, prepared from at least one plant selected from the group consisting of Blechnum orientale, Osmunda japonica, Woodwardia orientalis, Woodwardia unigemmata, Athyrium acrostichoides, Sphaeropteris lepifera, Cyrtomium falcatum and Cyrtomium fortunei;

c) LESPEDEZAE HERBA, prepared from at least one plant selected from the group consisting of Lespedeza cuneata and Senecio scandens;

d) POLYGONI CUSPIDATI RHIZOMA, prepared from the rhizome of at least one plant selected from the group consisting of Polygonum cuspidatum, Polygonum runcinatum and Polygonum reynoutria;

e) FORSYTHIAE FRUCTUS, prepared from the mature fruit of at least one plant selected from the group consisting of Forsythia suspensa, Forsythia viridissima and Forsythia koreana; and

f) LIGUSTRI FRUCTUS, prepared from at least one plant selected from the group consisting of Ligustrum lucidum and Ligustrum japonicum.

2. The composition of matter according to claim 1 additionally comprising at least one member selected from the group consisting of:

a) CIRSII RHIZOMA ET RADIX, prepared from the dried rhizoma or root or the whole plant of at least one plant selected from the group consisting of Cirsium japonicum, Cirsium albescens and Cirsium japonicum var. australe;

b) BREEAE RADIX, prepared from the dried root of at least one plant selected from the group consisting of Breea segetum and Breea setosum;

c) BAPHICACANTHIS RHIZOMA ET RADIX, prepared from the dried rhizoma and root of at least one plant selected from the group consisting of Baphicacanthes cusia, Strobilanthes cusia, Isatis tinctoria, Isatis indigotica and Polygonum tinctorium;

d) PHELLODENDRI CORTEX, prepared from the cortex of at least one plant selected from the group consisting of Phellodendron amurense, Phellodendron chinense, Phellodendron amurense var. sachalinense and Phellodendron wilsonii; and

e) BLETILLAE TUBER, prepared from the tuber of Bletilla striata.

3. A composition of matter useful in the treatment of viral infections, said composition comprising:

a) AEGINETIAE HERBA, prepared from the aqueous extract of the whole plant of at least one plant selected from the group consisting of Aeginetia indica, Dichondra micrantha, Striga lutea and Dichondra repens;

b) LONICERAE FLOS, prepared from the flower bud of at least one plant selected from the group

consisting of Lonicera japonica and Lonicera confusa;

c) PRUNELLAE SPICA, prepared from the spica or whole plant of at least one plant selected from the group consisting of Prunella vulgaris and Prunella vulgaris subsp. asiatica; and

d) LESPEDEZAE HERBA, prepared from at least one plant selected from the group consisting of Lespedeza cuneata and Senecio scandens.

4. The composition of matter according to claim 3, additionally comprising at least one member selected from the group consisting of:

a) SCUTELLARIAE, BARBATAE HERBA; and

b) FORSYTHIAE FRUCTUS.

Description

TECHNICAL FIELD

This invention relates to compositions of matter comprising the antiviral active components derived from Chinese herbal medicines, medicinal plants and extracts thereof, and to their use for the treatment of humans or animals infected with viruses, especially with hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). More specifically, the compositions of matter of the present invention are derived from various Chinese herbal medicines or medicinal plants which have a long history of human consumption. The compositions of matter of the invention are obtained through specific techniques and have demonstrated outstanding efficacy for treating human HBV carriers and hepatitis C patients. The compositions of matter according to the invention have also exhibited in vitro antiviral activities against murine leukemia virus (MuLV) and HIV. HIV is the virus known to cause acquired immunodeficiency syndrome (AIDS) in humans and AIDS presents special problems to the medical community which the present invention addresses. The active principles of the individual antiviral active herbal medicines or medicinal plants or extracts thereof have been isolated through specific isolation techniques and have been characterized through the use of accepted chemical techniques.

BACKGROUND

Modem medical science is constantly searching for new and more powerful agents to prevent, treat or retard bacterial and viral infections and cure the diseases they cause. Bacterial and viral infections of humans and domestic animals cost billions of dollars annually. Vast sums of money are spent each year by pharmaceutical companies to identify, characterize, and produce new antibiotics and antivirals to combat the emerging drug resistant strains which have become a serious problem. Reliable prophylactic treatments for disease prevention are also of major interest. Yet, despite the costs and efforts to identify treatments for viral infections, such as hepatitis and AIDS, effective therapies remain elusive.

Hepatitis is a disease of the human liver. It is manifested with inflammation of the liver and is usually caused by viral infections and sometimes from toxic agents. Hepatitis may progress to liver cirrhosis, liver cancer, and eventually death. Several viruses such as hepatitis A, B, C, D, E and G are known to cause various types of viral hepatitis. Among them, HBV and HCV are the most serious. HBV is a DNA virus with a virion size of 42 nm. HCV is a RNA virus with a virion size of 30-60 nm. See D. S. Chen, J.

Formos. Med. Assoc., 95(1), 6-12 (1996).

Hepatitis B is a major health problem worldwide, especially in Asia and Africa. Approximately 300 million people are chronically infected with HBV worldwide. More than one million carriers of HBV are found in the United States. HBV infection is currently the main cause of liver cirrhosis and cancer. HBV carriers are not only long-term reservoirs of the virus but also may develop chronic liver disease and have a greatly increased risk of developing liver cirrhosis and cancer. The progression from chronic hepatitis B to cirrhosis is frequently insidious and occurs without a noticeable change in symptoms. Once the symptoms of cirrhosis or cancer are manifested, therapies are of little value.

Prevention of HBV infection is possible through vaccination which is safe and effective. However, vaccination is not effective in treating those already infected, i.e., carriers and patients. Many drugs have been used in treating chronic hepatitis B and none have been proven to be effective, except interferon. Treatment with interferon has limited success and has frequently associated with adverse side effects such as fatigue, fever, chills, headache, myalgias, arthralgias, mild alopecia, psychiatric effects and associated disorders, autoimmune phenomena and associated disorders and thyroid dysfunction. Treatment with interferon for sixteen (16) weeks has been shown to be effective with a sustained loss of viral replication in approximately 40% of hepatitis B patients. The great majority of responders had normal serum aminotransferase levels and relapse rates appeared to be low. See R. P. Perrillo, Digestive Diseases and Sciences, 38(4), 577-593 (1993). However, a higher long-term relapse rate (24%) was reported in Chinese patients with chronic hepatitis B who underwent interferon therapy. See A. S. F. Lok, H. T. Chung, V. W. S. Liu, & O. C. K. Ma, Gastroenterology, 105(6), 1833-1838 (1993).

Moreover, serum hepatitis B surface antigen (HBsAg) disappeared in 10 to 15% of patients treated with interferon. The loss of HBsAg coincided with the disappearance of HBV. Improvement in liver histology was sustained years later in HBsAg-negative patients. The lack of disease progression could thus conceivably result in the prevention of liver cancer when treatment is provided in the pre-cirrhotic stage of infection. See R. P. Perrillo, Digestive Diseases and Sciences, 38(4), 577-593 (1993).

Hepatitis C has been previously described as a non-A non-B hepatitis, which is caused by HCV. There are approximately 100 million HCV carriers worldwide. An estimated 3.5 million people have chronic hepatitis C in the United States. HCV infection will lead to liver cirrhosis and cancer with less clinical manifestation. Most hepatitis C patients do not have particular symptoms and can thus be easily overlooked until it is too late for therapy. This poses a potentially more serious problem than hepatitis B. HCV carriers also become long-term reservoirs of the virus and eventually develop chronic liver disease and have a greatly increased risk of developing liver cirrhosis and cancer. See D. S. Chen, Science, 262, 369-370 (1993).

No effective immunization is currently available, and hepatitis C can only be controlled by preventive measures such as improvement in hygiene and sanitary conditions and interrupting the route of transmission. At present, the only acceptable treatment for chronic hepatitis C is interferon which requires at least six (6) months of treatment. Initial treatment has a response rate of about 50%. However, half of those responding relapse after cessation of interferon treatment. Therefore, only about 25% of patients have a sustained response. See D. S. Chen, J. Formos. Med Assoc., 95(1), 6-12 (1996) and N. Terrault & T. Wright, New Engl. J. Med., 332(22), 1509-1511 (1995). Because the interferon therapy has limited efficacy and frequent adverse effects, a more effective regimen is needed.

AIDS is a deadly disease caused by HIV. It has been plaguing the world since the first description of the disease in 1981 and the discovery of its causative agent, HIV, in 1983. About 13 million people were infected with HIV worldwide in 1993 and the number has increased to about 21 million in 1996. See B. Jasny, Science, 260(5112), 1219 (1993) and P. Piot, Science, 272(5270), 1855 (1996).

Several drugs have been approved for treatment of this devastating disease, such as azidovudine (AZT), didanosine (dideoxyinosine, ddI), d4T, zalcitabine (dideoxycytosine, ddC), nevirapine, lamivudine (epivir, 3TC), saquinavir (Invirase), ritonavir (Norvir), indinavir (Crixivan), and delavirdine (Rescriptor). See M. I. Johnston & D. F. Hoth, Science, 260(5112), 1286-1293 (1993) and D. D. Richman, Science, 272(5270), 1886-1888 (1996

All drugs currently approved for AIDS treatment utilize inhibition of viral proliferation and are viral reverse transcriptase inhibitors or viral protease inhibitors. More protease inhibitors, such as nelfinavir and improved saquinavir, are in development. An AIDS vaccine (Salk's vaccine) has been tested and several proteins which are chemokines from CD8 have been discovered to act as HIV suppressors.

In addition to the above synthetic nucleoside analogs, proteins, and antibodies, several plants and substances derived from plants have been found to have in vitro anti-HIV activity, such as Lonicera japonica and Prunella vulgaris, and glycyrrhizin from Glycyrrhiza radix. See R. S. Chang & H. W. Yeung, Antiviral Research, 9, 163-175 (1988) and M. Ito, et al., Antiviral Research, 7, 127-137 (1987).

Despite all of the available pharmaceuticals for the treatment of HIV, there is still no cure for the deadly disease. HIV viruses continue to mutate and become resistant to existing drugs such as the reverse transcriptase and protease inhibitors. Recently, a therapy of using two (2) or three (3) anti-HIV drugs in combination has been found effective in significantly lowering the HIV loads in AIDS patients. The results have been promising. However, the virus continues to develop resistance to the drugs and the long-term outcome (survival and cure rates) is still unknown. Thus, the medical communities throughout the world continue to search for drugs that can prevent the HIV infections, treat the HIV carriers to prevent them from progressing to full-blown deadly AIDS, and treat the AIDS patients.

Herbal Medicines

The use of herbal drugs and folk medicines have been known for thousands of years in China. These herbal approaches to the treatment of numerous illnesses, from arthritis to viral infections, have been previously viewed by western medicine as ineffective and dangerous. During the 19th century, many home remedies containing herbs were patented and sold. Modern drugs have replaced those remedies, but many modern drugs contain ingredients derived from herbs. For example, in 1776 the English botanist and physician William Withering learned that an herbal tea made by an old farm woman was effective in treating dropsy, or excess water in the tissues, which is caused by the inability of the heart to pump strongly enough. He found that one ingredient of the tea, which was made from leaves of the foxglove plant, strengthened the heart's pumping ability. The drug made from the foxglove plant is now known as digitalis.

Folk medicine is a relatively modern term to the West and has come to mean the care and treatment of the sick through a variety of herbal medicines. In recent years, folk medicines have become of increasing interest to many people in the western scientific medical community.

Prior Art--Herbal Medicines

A Chinese herbal medicine known as AEGINETIAE HERBA (a.k.a., GOLDEN LOCK KEY or LOTUS HERBA); has traditionally been used to treat illnesses such as swollen and sore throat, urinary tract infection, osteomyelitis, boil, tonsillitis, goiter, pharyngitis, thyroiditis, enteritis, liver disease, cancer, rheumatism, hematemesis, neurasthenia, eye redness, piles, menstruation irregularity, dropsy, jaundice, hernia, snake bite, and child developmental retardation. AEGINETIAE HERBA is prepared from the dried whole plant of Aeginelia indica which belongs to the family of Orobanchaceae. Dichondra

micrantha, Striga lutea and Dichondra repens are also used to prepare this herbal medicine. Treatment dosage using the dried plant is typically from 4 to 150 g per day. It should be noted that the plant tastes bitter and is toxic.

Okubo et al. disclose that a phosphate buffered saline (PBS) extract (pH 7.2 at ambient to 4.degree. C.) from the seeds of Aeginetia indica exhibits excellent carcinostatic effect and possesses interleukin-2 and interferon-.gamma. inducing potency. The PBS was a 0.1 M phosphate buffered physiological saline at pH 7.2, not containing calcium or magnesium ions. The extracted substance is taught to be a macromolecular polysaccharide which may or may not contain lipid A binding with protein depending on whether the extraction is conducted using butanol or phenol. The extracted substance was soluble in water and insoluble in n-butanol. Its molecular weight was within the range of 100,000 to 200,000 dalton. See S. Okubo, M. Sato, & K. Himeno, U.S. Pat. No. 5,366,725, issued on Nov. 22, 1994.

A Chinese herbal medicine known as BAPHICACANTHIS RHIZOMA ET RADIX has traditionally been used to treat numerous illnesses such as fever, abscess, erysipelas, swollen sore throat, headache, jaundice, plague, leucorrhea, and syphilis. BAPHITCACANTHIS RHIZOMA ET RADIX is prepared from the dried rhizoma and root of Baphicacanthes cusia, Strobilanthes cusia, Isatis tinctoria, Isatis indigotica, or Polygonum tinctorium. It has been reported that this herbal medicine has exhibited inhibition of flu virus in vitro. A decoction from boiling the root of Isatis tinctoria in water has also exhibited antibacterial effect. Baphicacanthes cusia and Strobilanthes cusia belong to the family of Acanthaceae. Isatis tinctoria and Isatis indigotica belong to the family of Cruciferae. Polygonum tinctorium belongs to the family of Polygonaceae. Treatment doses are typically 10 to 19 g per day for BAPHICACANTHIS RHIZOMA ET RADIX.

Ho et al. disclose the use of an extract from a mixture of herbs including Isatis tinctoria for the in vitro inhibition of HIV infection in human T lymphocyte cells and mononuclear phagocytic lineage cells. The activity was based on the test results of a water extract from a mixture of three herbs: Isatis tinctoria (or Isatis indigotica), Lonicera japonica, and Polygonum bistorta. See D. D. Ho & X. S. Li, U.S. Pat. No. 5,178,865, issued on Jan. 12, 1993.

A compound known as tryptanthrin has been identified as the principal antifungal agent in the leaf of Strobilanthes cusia and as the main antidermatophytic substance in the leaf of Polygonum tinctorium and Isatis tinctoria. See H. Y. Hsu, Y. P. Chen, & M. Hong, The Chemical Constituents Of Oriental Herbs, Vol. 2, Oriental Healing Arts Institute, Los Angeles, Calif., U.S.A., 758-759 (1985).

The Chinese herbal medicine known as BLECHNI RHIZOMA, which is also known as DRYOPTERIS CRASSIRHIZOMAE RHIZOMA has traditionally been used to treat illnesses such as cut injury, swelling, fever, measles and erysipelas. BLECHNI RHIZOMA is prepared from the dried root and stem of Blechnum orientate which belongs to the family of Polypodiaceae or Blechnaceae. DRYOPTERIS CRASSIRHIZOMAE RHIZOMA is prepared from the dried root and stem of Dryopteris crassirhizoma which belongs to the family of Aspidiaceae. Osmunda japonica (Osmundaceae family), Woodwardia orientalis and Woodwardia unigemmata (Blechnaceae family), Athyrium acrostichoides (Aspidiaceae or Athyriaceae family), Sphaeropteris lepifera (Cyatheaceae family), Cyrtomium falcatum, and Cyrtomium fortunei (Aspidiaceae family) have also been used for preparation of these herbal medicines. These herbal medicines taste bitter and astringent and are slightly toxic. Treatment dosages are typically 4 to 11 g per day.

Blechnum orientate has also shown a strong inhibition effect against the influenza virus. Filmarone, filicin, aspidin, albaspidin, and filicic acid which are found in Dryopteris crassirhizoma have been characterized as having an anthelmintic effect. See H. Y. Hsu, Y. P. Chen, S. G. Hsu, J. S. Hsu, C. J. Chen, & H. C. Chang, Concise Pharmacognosy, New Medicine Publishing Co., Taipei, R.O.C., 577-578

(1985); and H. Y. Hsu, Y. P. Chen, & M. Hong, The Chemical Constituents Of Oriental Herbs, Oriental Healing Arts Institute, Los Angeles, Calif., U.S.A., 249-250 (1982).

Hozumi et al. disclose the rhizome of Dryopteris crassirhizoma as an antiherpesviral agent, antipolioviral agent, and anti-varicella-zoster virus agent. The rhizome of Cyrtomium fortunei and the rhizome of Woodwardia orientalis are also disclosed as antiherpesviral, antipolioviral, anti-measles virus, anti-varicella-zoster virus, and anti-cytomegalovirus (CMV) agents, as well as an anti-DNA and anti-RNA virus agent. See T. Hozumi, T. Matsumoto, H. Ooyama, T. Namba, K. Shiraki, M. Hattori, M. Kurokawa, & S. Kadota, U.S. Pat. No. 5,411,733, issued May 2, 1995.

A Chinese herbal medicine known as BLETILLAE TUBER has traditionally been used to treat illnesses such as hemoptysis, epistaxis, hematemesis, abscess, burn, dry and chapped skin, tuberculosis, gastric ulcer, and sores. BLETILLAE TUBER has astringent, antibacterial and antifungal properties. BLETILLAE TUBER is prepared from the dried tuber of Bletilla striata which belongs to the family of Orchidaceae. BLETILLAE TUBER tastes bittersweet, astringent and is nontoxic. Treatment dose is typically 2 to 11 g per day for an average human.

Chinese herbal medicines known as CIRSII RHIZOMA ET RADIX and BREEAE RADIX have traditionally been used to treat illnesses such as hematemesis, acute infectious hepatitis, cut bleeding, sores, and abscess. CIRSII RHIZOMA ET RADIX is prepared from the dried rhizoma or root or the whole plant of plants such as Cirsium japonicum, Cirsium albescens, and Cirsium japonicum var. australe which are from the Compositae family. BREEAE RADIX is prepared from the dried root of Compositae family plants such as Breea segetum (also known as Cephalanoplos segetum) and Breea setosum. Both herbal medicines taste sweet and slightly bitter, and are nontoxic. Treatment dose is typically 5 to 75 g per day for the average human.

A Chinese herbal medicine known as DICHONDRAE HERBA has traditionally been used to treat illnesses such as jaundice, dysentery, gonorrhea, dropsy, swollen boil, convulsion, encephalitis, rheumatism, hernia, diabetes mellitus, and hypertension. DICHONDRAE HERBA is prepared from the dried whole plant of Dichondra repens or Dichondra micrantha which belongs to the family of Convolvulaceae. The plant tastes bitter and is nontoxic. Treatment dosage of the dried plant is typically 10 to 40 g per day. Nine (9) compounds which were isolated from n-hexane and ethanol extracts of the whole herb of Dichondra micrantha have been identified. These compounds are maltol, umbelliferone, scopoletin, umbelliferone-7-O-glucopyranoside, scopolin, astragalin, isoquercitrin, kaempferol-3-O-rutinoside, and quercetin-3-O-rutinoside. See C.-J. Chou, L.-C. Lin, S.-Y. Hsu and C.-F. Chen, J. Chin. Med., 4(2), 143-149 (1993).

A Chinese herbal medicine known as FORSYTHIAE FRUCTUS has traditionally been used to treat illnesses such as sores, abscess, lymph node swelling, urethritis, and hypertension. It was also found to inhibit several bacteria and influenza virus. FORSYTHIAE FRUCTUS is prepared from the dried mature fruit of Forsythia suspensa, Forsythia viridissima, or Forsythia koreana which belong to the family Oleaceae. The herbal medicine tastes bitter and is nontoxic. Treatment dosage is typically 3 to 11 g per day.

Hozumi et al. disclose that the fruit of Forsythia suspensa is an antipolioviral agent and an anti-measles virus agent useful in treating these viral infections. See T. Hozumi, T. Matsumoto, H. Ooyama, T. Namba, K. Shiraki, M. Hattori, M. Kurokawa, & S. Kadota, U.S. Pat. No. 5,411,733, issued May 2, 1995.

The compounds forsythoside A (found in the leaf of Forsythia suspensa), forsythoside B (found in the stem of Forsythia koreana), and forsythoside C and forsythoside D (found in the fruit of Forsythia

suspensa) have been reported to exhibit antibacterial activity against Staphylococcus aureus at a concentration less than 2 mM. Suspensaside (found in the fruit of Forsythia suspensa, likely the same as forsythoside C) has also been reported to exhibit antibacterial activity against Staphylococcus aureus Terashima with a minimum inhibition concentration (MIC) of 2.6 mg/mL. See H. Y. Hsu, T. P. Chen & M. Hong, The Chemical Constituents of Oriental Herbs, Vol. 2, Oriental Healing Arts Institute, Los Angeles, Calif., U.S.A., 53-55, 142-143 (1985).

A Chinese herbal medicine known as HEDYOTIS (also known as OLDENLANDIAE HERBA) has traditionally been used to treat illnesses such as urethra infection, pharyngitis, laryngitis, tonsillitis, subacute or chronic coccygodynia, appendicitis, intestinal cancer, contusion injury and eye disease. It has also been found to have weak antibacterial activity in vitro. HEDYOTIS is prepared from the dried whole plant of Hedyotis diffusa (also known as Oldenlandia diffusa) which belongs to the family Rubiaceae. The herbal medicine tastes sweet and is nontoxic. Treatment dosage is typically 19 to 300 g per day.

The Chinese herbal medicines known as LESPEDEZAE HERBA and SENECINIS HERBA have traditionally been used to treat illnesses such as urine incontinence, gonorrhea, asthma, stomach ache, general weakening and exhaustion, diarrhea, contusion injury, eye disease, eye redness, renal disease, acute inflammatory disease, cataract, dysentery, enteritis, jaundice, flu, septicemia, sore, swelling, and a disease of the palm. LESPEDEZAE HERBA is prepared from the dried whole plant of Lespedeza cuneata which belongs to the family Leguminosae. SENECINIS HERBA is prepared from the dried whole plant of Senecio scandens which belongs to the family Compositae. The extracts of Lespedeza cuneata and Senecio scandens have been shown to have an antibacterial effect. Both herbs taste sour, astringent and bitter. Treatment dose is typically 4 to 40 g per day.

A Chinese herbal medicine known as LIGUSTRI FRUCTUS has traditionally been used as a tonic and to treat illnesses such as insomnia, constipation, early white hair, neck lymph nodes tuberculosis and dropsy. LIGUSTRI FRUCTUS is prepared from the dried mature fruit of Ligustrum lucidum or Ligustrum japonicum which belongs to the family Oleaceae. The leaves of Ligustrum lucidum have been used as an antipyretic, analgesic, and anti-inflammatory agent. The leaves of Ligustrum japonicum have also been used to treat illnesses such as ophthalmalgia, ulcerative stomatitis, mastitis, swelling, and bum. The fruits of Ligustrum lucidum taste bitter and are nontoxic. Treatment dosage of the dried fruits is typically 6 to 20 g per day. That of the dried leaves is typically 40 to 75 g per day.

A Chinese herbal medicine known as LONICERAE FLOS has traditionally been used to treat illnesses such as fever, acute infectious diseases, measles, carbuncle, dysentery, enteritis, ringworm and similar skin diseases. LONICERAE FLOS is prepared from the dried flower bud of Lonicera japonica or Lonicera confusa. Both plants belong to the family Caprifoliaceae. The flower of Lonicera japonica has diuretic, antipyretic, anti-inflammatory, anti-convulsive, antibacterial and antiviral properties. The flower bud has also been used as a diuretic. The herbal medicine tastes sweet and is nontoxic. Treatment dosage is typically 11 to 75 g per day for the typical human.

Ho et al. disclose the anti-HIV activity in vitro of a mixture of Lonicera japonica, Isatis tinctoria (or Isatis indigotica) and Polygonum bistorta or a mixture of Lonicera japonica with Scutellaria baicalensis. Water extractions of the mixtures, treatment with ethanol precipitation and charcol adsorption are disclosed for the preparation of the anti-HIV active composition. See D. D. Ho & X. S. Li, U.S. Pat. No. 5,178,865, issued on Jan. 12, 1993. Several tannins such as caffeoylquinates isolated from Lonicera japonica have been reported to have an inhibitory effect on HIV-1 reverse transcriptase activity. See C.-W. Chang, M.-T. Lin, S.-S. Lee, K. C. S. C. Liu, F.-L. Hsu, & J.-Y. Lin, Antiviral Research, 27, 367-374 (1995).

A mixture of aqueous extracts of Lonicera japonica flower buds and Forsythia suspensa fruits with the crude flavonoids from Scutellaria baicalensis has been shown to have antibacterial and antiviral properties. A group of patients with severe respiratory disease were treated with the mixture and they responded as well as a control group on standard antibiotic therapy. See P. J. Houghton, Z. Boxu, & Z. Xisheng, Phytother. Res., 7, 384-386 (1993).

A Chinese herbal medicine known as PHELLODENDRI CORTEX has traditionally been used to treat illnesses such as dysentery, diarrhea, jaundice, stools with blood, abdominal pain, indigestion, bacteroid enteritis, and tuberculoid diarrhea. The herbal medicine has also been used to prepare an eye wash, for strengthening stomach and intestine to stimulate appetite, and as an astringent, anti-inflammatory, etc. It has antibacterial, anti-inflammatory, and wound healing properties. PHELLODENDRI CORTEX is prepared from the dried cortex of plants from the Rutaceae family such as Phellodendron amurense, Phellodendron chinense, Phellodendron amurense var. sachalinense, and Phellodendron wilsonii. PHELLODENDRI CORTEX tastes bitter and is nontoxic. Treatment dose is typically 1 to 11 g per day.

Hozumi et al. disclose the bark of Phellodendron amurense as antiherpesviral, antipolioviral, anti-measles virus, anti-varicella-zoster virus, and anti-CMV agents, as well as an anti-DNA virus and anti-RNA virus agent. See T. Hozumi, T. Matsumoto, H. Ooyama, T. Namba, K. Shiraki, M. Hattori, M. Kurokawa, & S. Kadota, U.S. Pat. No. 5,411,733, issued on May 2, 1995.

A Chinese herbal medicine known as POLYGONI CUSPIDATI RHIZOMA has traditionally been used to treat illnesses such as dysentery, menorrhagia, dysmenorrhea, dysuria, infantile growth retardation, and appendicitis. POLYGONI CUSPIDATI RHIZOMA is prepared from the dried rhizoma of Polygonum cuspidatum, Polygonum runcinatum, or Polygonum reynoutria (also known as Reynoutria japonica) which belong to the family Polygonaceae. The tender leaf has also been used to treat contusion and cut injuries. Extract of the herbal medicine has exhibited antibacterial and antiviral effects in vitro. Excessive use of the herbal medicine may cause a slight diarrhea. The herbal medicine tastes bitter and the treatment dose is typically 6 to 40 g per day.

Hozumi et al. disclose the root and rhizome of Polygonum cuspidatum as an antiherpesviral, antipolioviral, anti-varicella-zoster virus, and anti-CMV agent. See T. Hozumi, T. Matsumoto, H. Ooyama, T. Namba, K. Shiraki, M. Hattori, M. Kurokawa, & S. Kadota, U.S. Pat. No. 5,411,733, issued on May 2, 1995.

Resveratrol has been reported as an antifungal and antibacterial component in the root of Polygonum cuspidatum. See H. Y. Hsu, Y. P. Chen, & M. Hong, The Chemical Constituents Of Oriental Herbs, Vol. 2, Oriental Healing Arts Institute, Los Angeles, Calif., U.S.A., 51 (1985).

A Chinese herbal medicine known as PRUNELLAE SPICA has traditionally been used to treat illnesses such as goiter, hemorrhoids, swollen eye, ophthalmalgia, gonorrhea, uterine disease, mastitis, breast abscess, breast cancer, chronic arthritis, conjunctivitis, and hypertension. PRUNELLAE SPICA is prepared from the dried spica or whole plant of Prunella vulgaris or Prunella vulgaris subsp. asiatica (also known as Prunella vulgaris var. lilachina). Both plants belong to the family Labiatae. The whole plant can be used as a diuretic and also has antibacterial effect in vitro. The herbal medicine tastes bitter and is nontoxic. Treatment dosage is typically 4 to 110 g per day for the average human.

Hozumi et al. disclose the spike of Prunella vulgaris as an antiherpesviral agent for treating herpes virus infection. See T. Hozumi, T. Matsumoto, H. Ooyama, T. Namba, K. Shiraki, M. Hattori, M. Kurokawa, & S. Kadota, U.S. Pat. No. 5,411,733, issued May 2, 1995. The water extract of Prunella vulgaris (boiling 3 g in 100 mL water for 45 minutes) has also been reported to have anti-HIV (strain H9/3B) activity. The extract also exhibited synergistic anti-HIV activity with zidovudine (AZT) and didanosine

(ddI). Only a slight additive effect was observed for Prunella vulgaris and zalcitabine (ddC). See J. F. John, R. Kuk, & A. Rosenthal, Abstr. Gen. Meet. Am. Soc. Microbiol., 94, 481(1994).

Yamasaki et al. evaluate in vitro two hundred four (204) crude drugs of common use in Japan for anti-HIV-1 activity and reported that the hot water extract of Prunella vulgaris (spike) showed a strong in vitro anti-HIV-1 activity with an IC.sub.100 of 16 .mu.g/mL. See K. Yamasaki, T. Otake, H. Mori, M. Morimoto, N. Ueba, Y. Kurokawa, K. Shiota, & T. Yuge, Yakugaku Zasshi, 113(11), 818-824 (1993).

Yao et al. reported that the water extract of the dried entire plant of Prunella vulgaris was active in vitro in inhibiting HIV-1 replication with relatively low cytotoxicity towards the MT-4 cells. The extract was also active in reverse transcriptase inhibition. The active factor was purified and identified as anionic with a molecular weight of approximately 10,000 dalton. This active component may be the same as the prunellin, as described below by Tabba, et al. The purified extract inhibited HIV-1 replication in the lymphoid cell line MT-4, in the monocytoid cell line U937, and in peripheral blood mononuclear cells (PBMC) at effective concentrations of 6, 30, and 12.5 .mu.g/mL, respectively. Pretreatment of uninfected cells with the extract prior to viral exposure did not prevent HIV-1 infection. Preincubation of HIV-1 with the purified extract dramatically decreased infectiousness. The purified extract was also able to block cell-to-cell transmission of HIV-1, prevented syncytium formation, and interfered with the ability of both HIV-1 and purified gp120 to bind to CD4. PCR (polymerase chain reaction) analysis confirmed the absence of HIV-1 proviral DNA in cells exposed to virus in the presence of the extract. The results suggested that the purified extract antagonized HIV-1 infection of susceptible cells by preventing viral attachment to the CD4 receptor. See X. J. Yao, M. A. Wainberg, & M. A. Parniak, Virology, 187(1), 56-62 (1992).

Tabba et al. isolated and partially characterized an anti-HIV component, prunellin, from aqueous extracts of dried inflorescence of Prunella vulgaris. Prunellin is a carbohydrate with an MIC (minimum inhibition concentration) of 2.2 .mu.g/mL against HIV-1 in vitro. It was identified as a partially sulfated polysaccharide with a molecular weight of about 10,000 dalton. See H. D. Tabba, R. S. Chang, & K. M. Smith, Antiviral Research, 11, 263-273 (1989).

Zheng evaluated four hundred seventy two (472) traditional medicinal herbs for antiviral effect on type 1 herpes simplex virus (HSV 1). Prunella vulgaris was one of the ten herbs found to be highly effective in vitro. Clinically, 78 cases of herpetic keratitis due to HSV1 were treated with Prunella vulgaris and Pyrrosia lingua eye drops. Among them, 38 cases were effectively cured, 37 cases showed an improvement, and 3 cases showed no benefit. See M. Zheng, J. Tradit. Chin. Med., 8(3), 203-206 (1988).

Triterpene 1 and triterpene 2 which have been isolated from Prunella vulgaris have shown antiviral activity against HSV1. Triterpene 1 was identified as betulinic acid and triterpene 2 was identified as 2.alpha., 3.alpha.-dihydroxyurs-12-en-28-oic acid. The EC.sub.50 was estimated to be 30 .mu.g/mL for triterpene 1 and 8 .mu.g/mL for triterpene 2 by plaque reduction assay. See S. Y. Ryu, C-K. Lee, C. O. Lee, H. S. Kim, & O. P. Zee, Arch. Pharmacal Res. (Seoul), 15(3), 242-245 (1992).

A Chinese herbal medicine known as SCUTELLARIAE BARBATAE HERBA has traditionally been used to treat illnesses such as hematemesis, gonorrhea with traces of blood, sores, cancer, convulsion, pneumonia, enteritis, coccygodynia, appendicitis, asthma, malaria, and rheumatism. It was also found to have antibacterial effect. SCUTELLARIAE BARBATAE HERBA is prepared from the dried whole plant of Scutellaria barbata, Scutellaria rivularis, or Scutellaria dependens which belong to the family Labiatae. The herbal medicine tastes bitter and should not be consumed by those who have anemia. Pregnant women should avoid taking this herb. Treatment dosage is typically 4 to 300 g per day.

Dried whole plants of Scutellaria rivularis have been used in folk medicine for the treatment of tumors, hepatitis, liver cirrhosis, and other diseases in China and Taiwan. See Y. L. Lin, Y. H. Kuo, G. H. Lee, and S. M. Peng, J. Chem. Research (S), 320-321 (1987).

Apigenin, isolated from the whole herb of Scutellaria rivularis, was found to have anti-influenza virus activity. See T. Nagai, et al., Chem. Pharm. Bull., 38(5), 1329-1332 (1990).

A Chinese herbal medicine known as SOLANI HERBA has traditionally been used to treat illnesses such as boil, acute nephritis, cancer and sores. SOLANI HERBA is prepared from the dried whole plant of Solanum nigrum which belongs to the family Solanaceae. The extract of SOLANI HERBA has demonstrated anti-inflammatory property. The fruit has also exhibited the effects of suppressing coughs and relieving bronchial inflammation. The herbal medicine tastes bitter and slightly sweet, and is nontoxic. Treatment dosage is typically 11 to 60 g per day.

The compound solasonine (found in the whole herb, fruit, leaf, and fresh immature berries of Solanum nigrum) has an anti-inflammatory effect similar to cortisone. Solasonine and solanine (also found in Solanum nigrum) possess the ability of raising or lowering the blood sugar level in rats depending on the situation of the animals. Solasonine was also reported to have a stimulating effect on the heart, while solanine had a suppressive effect. When administered at small doses, solasonine enhances the stimulative process of the central nerve system in animals (i.e., rat and rabbit). On the other hand, it enhances the suppressive process when administered at large doses. Solasonine can also lower the blood coagulability. See (1) H. Y. Hsu, Y. P. Chen, S. G. Hsu, J. S. Hsu, C. J. Chen, & H. C. Chang, Concise Pharmacognosy, New Medicine Publishing Co., Taipei, R.O.C., 176-177 (1985); (2) H. Y. Hsu, Y. P. Chen, & M. Hong, The Chemical Constituents Of Oriental Herbs, Oriental Healing Arts Institute, Los Angeles, Calif., U.S.A., 1400-1401, 1406 (1982); and (3) H. Y. Hsu, Y. P. Chen, & M. Hong, The Chemical Constituents Of Oriental Herbs, Vol. 2, Oriental Healing Arts Institute, Los Angeles, Calif., U.S.A., 742 (1985).

Combinations of herbal medicines such as LONICERAE FLOS, BAPHICACANTHIS RHIZOMA ET RADIX, and FORSYTHIAE FRUCTUS have been used as antipyretic and detoxification agents and for treating acute hepatitis. The herbal medicines BLECHNI RHIZOMA and POLYGONI CUSPIDATI RHIZOMA have been used along with other herbal medicines in a formula for treating Hepatitis B. The herbal medicines SCUTELLARIAE BARBATAE HERBA and LIGUSTRI FRUCTUS have occasionally been added with other herbal medicines into the above formula to improve the treatment. The herbal medicine LIGUSTRI FRUCTUS was occasionally used along with other herbal medicines mainly as a tonic and the herbal medicine HEDYOTIS has been occasionally used along with other herbal medicines as a detoxification agent. The herbal medicine PRUNELLAE SPICA has also been used along with other herbal medicines to relief liver stress.

Chang and Yeung screened the boiling water extracts of twenty seven (27) medicinal herbs for anti-HIV activity. They found eleven (11) of the extracts were active in inhibiting HIV in the H9 cells. Lonicera japonica, Prunella vulgaris, Woodwardia unigemmata, and Senecio scandens were among those active ones with moderate activities. Forsythia suspensa, Isatis tinctoria, and Polygonum cuspidatum were among those tested which did not display activity in the anti-HIV assay. The anti-HIV active extract of Viola yedoensis was further tested and found to be fairly specific. The extract did not inactivate HIV extracellularly and did not inhibit the growth of herpes simplex, polio, or vesicular stomatitis viruses in human fibroblast culture. See R. S. Chang & H. W. Yeung, Antiviral Research, 9, 163-175 (1988).

Antiviral agents have been isolated from Syzygium aromaticum, Sapium sebiferum, Scutellaria baicalensis, and Scutellaria rivularis. Eugeniin (a tannin) isolated from Syzygium aromaticum and methyl gallate isolated from Sapium sebiferum exhibited anti-herpes simplex virus activity in vitro.

Plant flavenoids, such as 5,7,4'-trihydroxy-8-methoxyflavone from the root of Scutellaria baicalensis and apigenin (5,7,4'-trihydroxyflavone) from the whole herb Scutellaria rivularis, were also reported to have anti-influenza virus activity. See (1) T. Hozumi, et al., U.S. Pat. No. 5,411,733 (1995); (2) M. Takechi & Y. Tanaka, Planta Medica, 42, 69-74 (1981); (3) C. J. M. Kane, et al, Bioscience Reports, 8, 85-94 (1988); and (4) T. Nagai, et al., Chem. Pharm. Bull., 38(5), 1329-1332 (1990).

Hozumi et al. investigated ninety one (91) herbal medicines which demonstrated antiviral activity. More specifically, fifty two (52) of them had antiherpesviral activity, sixty four (64) had antipolioviral activity, thirty seven (37) had anti-measles virus activity, twenty seven (27) had anti-varicella-zoster virus activity, twenty three (23) had anti-CMV activity, and twenty eight (28) had anti-DNA virus and anti-RNA virus activity. See T. Hozumi, T.

Matsumoto, H. Ooyama, T. Namba, K. Shiraki, M. Hattori, M. Kurokawa, & S. Kadota, U.S. Pat. No. 5,411,733, issued on May 2, 1995. The anti-DNA virus and anti-RNA virus activity of the twenty eight (28) herbal medicines disclosed in the 5,411,733 patent was based upon their antiherpesviral, antipolioviral, anti-measles virus, and/or anti-varicella-zoster virus and anti-CMV activities. However, the extrapolation to cover both anti-DNA virus and anti-RNA virus activities is unfounded from the experiments conducted.

The data of the present invention, presented below, evidenced little or no anti-HIV activity of the two herbal medicines at 2.5 and 0.5 mg/mL derived from the rhizome of Cyrtomium fortunei and the bark of Phellodendron amurense. In contrast, the three (3) herbal medicines using the spike of Prunella vulgaris, the fruit of Forsythia suspensa, and the root and rhizome of Polygonum cuspidatum will be shown to have a strong to moderate anti-HIV activity at 2.5 mg/mL. Prunella vulgaris has also been reported by others as described above to have a very good anti-HIV activity.

It is noted that in the practice of traditional Chinese medicine, herbal medicines are used to treat the symptoms of the patients, not the disease or causative agent itself, and are therefore not known to be specific to a particular disease. Herbal medicines were prescribed depending on the symptoms of the individual patient. The composition of herbal medicines also vary case by case and may even change for each individual patient during the course of the treatment according to each treatment result. It is therefore very difficult to describe a particular herbal composition from the prior art suitable for treating a specific disease.

The present invention is directed to the discovery of antiviral herb compositions, extracts thereof and the active chemical constituents thereof. The antiviral herb compositions of this invention are derived from individual herbs, herb mixtures and commercially available Chinese herbal medicines. These novel herb compositions and their extracts and/or active principles are demonstrated herein as active against viral diseases such as HBV and HCV carriers, hepatitis B, hepatitis C, HIV infection and AIDS.

SUMMARY OF THE INVENTION

As used herein and in the claims, the following nomenclatures will be used to identify the four (4) herb mixtures known as HHT888-4, HHT888-5, HHT888-45 and HHT888-54.

HHT888-4 is a mixture of five (5) single-herb Chinese herbal medicines at a preferred ratio of No.4 (1):No.4(2):No.4(3):No.4(4):No.4(5) of about 3:3:3:3:4 (w/w). The weight ratio may vary up to 50% per component. By "variance of the weight ratio by 50%" means that each value of each component of the ratio may be increased or decreased by 50%. Thus, as an example, 1:1 can range from 1.5:0.5to 0.5:1.5 (or3:1 to 1:3).

HHT888-5 is a mixture of eleven (11) single-herb Chinese herbal medicines, No.5(1) to No.5(11), preferably at about equal proportions by weight. The weight ratio may vary up to 50% per component.

HHT888-45 is a mixture of four (4) to six (6) single-herb Chinese herbal medicines at a preferred ratio of No.4(3): No.4(4): No.5(4): No.5(5): No.5(8): No.4(2) of about 1:1:1:1:0-1:0-1 (w/w). The weight ratio may vary up to 50% for each component.

HHT888-54 is a mixture of No.5(5) or H and at least one single-herb medicine selected from No.4(2), No.4(3), No.4(4), No.4(5), No.5(1), No.5(2), No.5(4), No.5(7), No.5(8) and No.5(11), wherein the preferred weight ratio of No.5(5) or H to each of the other single-herb medicines is 1:1. Thus, HHT888-54, in a preferred embodiment, consists of No.5(5) or H plus No.4(3), No.4(4), No.5(8) and No.5(11); the preferred weight ratio is 1:1:1:1:1. More generally, the weight ratio of No.5(5) or H to the sum of the other single-herb medicines is from 1:10 to 10:1.

The single-herb components of HHT888-4 are:

No.4(1)=HEDYOTIS (also known as OLDENLANDIAE HERBA) source: Hedyotis diffusa (also known as Oldenlandia diffusa)

No.4(2)=SCUTELLARIAE BARBATAE HERBA source: Scutellaria barbata, Scutellaria rivularis, Scutellaria dependens

No.4(3)=LONICERAE FLOS source: Lonicera japonica, Lonicera confusa

No.4(4)=PRUNELLAE SPICA source: Prunella vulgaris, Prunella vulgaris subsp. asiatica (also known as Prunella vulgaris var. lilachina)

No.4(5)=SOLANI HERBA source: Solanum nigrum

The single-herb components of HHT888-5 are:

No.5(1)=HEDYOTIS (also known as OLDENLANDIAE HERBA) source: Hedyotis diffusa (also known as Oldenlandia diffusa)

No.5(2)=BLECHNI RHIZOMA or DRYOPTERIS CRASSIRHIZOMAE RHIZOMA source: Blechnum orientale, Dryopteris crassirhizoma, Osmunda japonica, Woodwardia orientalis, Woodwardia unigemmata, Athyrium acrostichoides, Sphaeropteris lepifera, Cyrtomium falcatum, Cyrtomium fortunei

No.5(3)=CIRSII RHIZOMA ET RADIX and BREEAE RADIX source: Cirsium japonicum, Cirsium albescens, Cirsium japonicum var. australe, Breea segetum (also known as Cephalanoplos segetum), Breea setosum

No.5(4)=LESPEDEZAE HERBA or SENECINIS HERBA source: Lespedeza cuneata, Senecio scandens

No.5(5)=AEGINETIAE HERBA (a.k.a GOLDEN LOCK KEY or LOTUS HERBA). source: Aeginetia indica Dichondra micrantha, Striga lutea Dichondra repens.

No.5(6)=BAPHICACANTHIS RHIZOMA ET RADIX source: Baphicacanthes cusia, Strobilanthes cusia, Isatis tinctoria, Isatis indigotica, Polygonum tinctorium

No.5(7)=POLYGONI CUSPIDATI RHIZOMA source: Polygonum cuspidatum, Polygonum runcinatum, Polygonum reynoutria (also known as Reynoutria japonica)

No.5(8)=FORSYTHIAE FRUCTUS source: Forsythia suspensa, Forsythia viridissima, Forsythia koreana

No.5(9)=PHELLODENDRI CORTEX source: Phellodendron amurense, Phellodendron chinense, Phellodendron amurense var. sachalinense, Phellodendron wilsonii

No.5(10)=BLETILLAE TUBER source: Bletilla striata

No.5(11)=LIGUSTRI FRUCTUS source: Ligustrum lucidum, Ligustrum japonicum

The single-herb components of HHT888-45 are:

No.4(3)=LONICERAE FLOS source: Lonicera japonica, Lonicera confusa

No.4(4)=PRUNELLAE SPICA source: Prunella vulgaris, Prunella vulgaris subsp. asiatica (also known as Prunella vulgaris var. lilachina)

No.5(4)=LESPEDEZAE HERBA or SENECINIS HERBA source: Lespedeza cuneata, Senecio scandens

No.5(5)=AEGINETIAE HERBA source: Aeginetia indica Dichondra micrantha, Striga lutea, Dichondra repens.

No.4(2)=SCUTELLARIAE BARBATAE HERBA (optional) source: Scutellaria barbata, Scutellaria rivularis, Scutellaria dependens

No.5(8)=FORSYTHIAE FRUCTUS (optional) source: Forsythia suspensa, Forsythia viridissima, Forsythia koreana

The single-herb components of HHT888-54 are: in addition to No. 5(5) are at least one selected from:

No.5(5)=AEGINETIAE HERBA (a.k.a. GOLDEN LOCK KEY or LOTUS HERBA). source: Aeginetia indica; or

H=DICHONDRAE HERBA source: Dichondra repens or Dichondra micrantha; and at least one selected from:

No.4(2)=SCUTELLARIAE BARBATAE HERBA source: Scutellaria barbata, Scutellaria rivularis, Scutellaria dependens

No.4(3)=LONICERAE FLOS source: Lonicera japonica, Lonicera confusa

No.4(4)=PRUNELLAE SPICA source: Prunella vulgaris, Prunella vulgaris subsp. asiatica (also known as Prunella vulgaris var. lilachina)

No.4(5)=SOLANI HERBA source: Solanum nigrum

No.5(1)=HEDYOTIS (also known as OLDENLANDIAE HERBA) source: Hedyotis diffusa (also known as Oldenlandia diffusa)

No.5(2)=BLECHNI RHIZOMA or DRYOPTERIS CRASSIRHIZOMAE RHIZOMA source: Blechnum orientale, Dryopteris crassirhizoma, Osmunda japonica, Woodwardia orientalis, Woodwardia unigemmata, Athyrium acrostichoides, Sphaeropteris lepifera, Cyrtomium falcatum, Cyrtomium fortunei

No.5(4)=LESPEDEZAE HERBA or SENECINIS HERBA source: Lespedeza cuneata, Senecio scandens

No.5(7)=POLYGONI CUSPIDATI RHIZOMA source: Polygonum cuspidatum, Polygonum runcinatum, Polygonum reynoutria (also known as Reynoutria japonica)

No.5(8)=FORSYTHIAE FRUCTUS source: Forsythia suspensa, Forsythia viridissima, Forsythia koreana

No.5(11)=LIGUSTRI FRUCTUS source: Ligustrum lucidum, Ligustrum japonicum

It should be noted that No.4(1) is the same as No. 5(1) (HEDYOTIS). The names of the Chinese herbal medicines for the single-herb components are shown in capital letters, followed by their plant sources listed in italics.

As used herein and in the claims, the term HHT888-4, HHT888-5, HHT888-45 and HHT888-54 include the actual herbal blends, aqueous extracts thereof and the active components or principles of the extract. In similar fashion, the use of the terms No.5(5), No.5(8) and the like include the actual herb, extracts thereof and the isolated active molecular agents.

As also used in the specification and in the claims, G is the herb Aeginetia indica or a source plant of No.5(5). No.4(2), No.4(3), No.4(4), No.4(5), No. 5(1), No.5(2), No.5(3), No.5(4), No.5(5), No.5(6), No.5(7), No.5(8), No.5(9), No. 5(10), No.5(11) and H are the single-herb components described above, including their respective source plants.

Specific descriptions of the above recited Chinese herbal medicines and medicinal herbs can be found in the following references: (1) H. C. Chang, Medicinal Herbs I, Holiday Publishing Co., Taipei, Taiwan, R.O.C., 15, 36, 100, 113, 127, 147 (1990); (2) H. C. Chang, Medicinal Herbs II, Holiday Publishing Co., Taipei, Taiwan, R.O.C., 15, 27, 131, 135, 155 (1991); (3) W. S. Kan, Pharmaceutical Botany, National Research Institute Of Chinese Medicine, Taipei, Taiwan, R.O.C., 113, 124-130, 200-201, 206-207, 289-290, 353-354, 442-444, 460-461, 485, 487-488, 497, 505, 513-514, 522, 527-529, 558, 562-563, 648-649 (1971); (4) M. S. Lee, Frequently Used Chinese Crude Drugs And Folk Medicines Handbook, 12th Ed., Sheng-Chang Medicinal Record Magazine Publishing Co., Taipei, Taiwan, R.O.C., 4-6, 17, 21, 29, 36, 38, 40, 48, 58, 64, 71, 79, 85 (1992); and (5) H. Y. Hsu, Y. P. Chen, S. G. Hsu, J. S. Hsu, C. J. Chen, & H. C. Chang, Concise Pharmacognosy, New Medicine Publishing Co., Taipei, Taiwan, R.O.C., 90, 97, 105-106, 117-118, 126-127, 130-131, 133, 138, 144-145, 152-153, 156-157, 161-162, 174, 176-177, 357-358, 381-382, 384-385, 456-457, 577-578 (1985).

The present invention in its broadest aspect relates to the described herbal medicine mixtures and their use to prevent and treat viral infections. The invention also relates to novel combinations of medicinal herbs and the herbal medicines derived therefrom. For example, the herbal mixtures designated HHT888-4, HHT888-5, HHT888-45, HHT888-54, No.5(5)-H, No.5(5)-No.4(3), No.5(5)-No.4(4), No.4(3)-No. 4(4), No.5(5)-No.5(11), H-No.4(4), H-No.4(3), H-No.4(5), H-No.5(8), H-No.5(11), mixtures

thereof and their pharmaceutically acceptable salts and the like. More specifically, the viral infections are those caused by HBV, HCV and HIV. The antiviral mixtures according to the invention have been described above as HHT888-4, HHT888-5, HHT888-45 and HHT888-54. In addition, the single herb agents designated No.4(2), No.4(5), No.5(5), No.5(7), No.5(8), No.5(11) and H have been shown to have antiviral activity. These single herb agents have not been shown by the prior art to have antiviral activity.

A more specific aspect of the present invention resides in the discovery that HHT888-5 is efficacious in reducing hepatitis B viruses in HBV carriers. An additional aspect of the invention resides in the discovery that HHT888-45 is efficacious in treating hepatitis C patients and returning their liver function to normal.

The herb mixtures HHT888-4 and HHT888-5 and their aqueous extracts have both been shown by the inventors herein to also have antiretroviral activities against MuLV and HIV in vitro. This evidence strongly supports the conclusion that they have in vivo efficacy. In addition, eleven (11) of the fifteen (15) single-herb components of HHT888-4 and HHT888-5, i.e., No.4(2), No.4(3), No.4(4), No.4(5), No.5(1), No. 5(2), No.5(4), No.5(5), No.5(7), No.5(8), No.5(11), and the medicinal herb H have shown anti-HIV activities by effectively suppressing viral proliferation in HIV infected human peripheral blood lymphocytes (PBLs). This model is highly predictive of anti-HIV activity in vivo.

The water extract of the single-herb component No.5(5) prepared directly from its source plant, Aeginetia indica, has shown good anti-HIV activity. Further, the water extracts of the single-herb components No.4(3), No.4(4), and No.5(11) have shown moderate to strong anti-HIV activities. Water extracts of the single-herb components No.4(2), No.4(5), No.5(1), No.5(4) and No.5(8) have shown only weak anti-HIV activities.

The water extractable and acid precipitable components of No.4(2), No.4(5), No.5(1), No.5(5), No.5(8) and H are shown herein to be active anti-HIV agents. Similar water extractable and acid precipitable components have also been isolated from No.4(4) and No.5(11) and are shown herein to be anti-HIV. These water extractable and acid precipitable anti-HIV active components are similar, have not been described before, and are novel and unobvious.

Water extractable and acid soluble anti-HIV active components have also been isolated from No.4(4) and No.5(11). The water extractable and acid soluble active component of No.5(11) has not been described before and is novel. The water extractable and acid soluble active component of No.4(4) may be the same as the partially sulfated polysaccharide or prunellin described before. Only one active component has been isolated from the water extract of No.4(3) which is soluble in acid.

There is further disclosed as compositions of matter, the herb mixtures HHT888-4, HHT888-5, HHT888-45 and HHT888-54. These compositions of matter have not been described before and are unobvious.

Further disclosed is a composition of matter comprising at least one of the individual water extractable and acid precipitable anti-HIV active components isolated separately or in combination from the single-herb herbal medicines or their source plants selected from the group consisting of: No.4(2), No.4(4), No.4(5), No.5(1), No.5(5), No.5(8), No.5(11) and H. These compositions of matter have not been described before and are unobvious and novel.

The utility of the present compositions of matter resides in their use in treating viral infections. Thus, there is further disclosed a method of treating viral infections in a mammal, said method comprising administering to said mammal a therapeutically effective amount (such as from 0.4 to 120 g per day) of

at least one composition selected from the group consisting of HHT888-4, HHT888-5, HHT888-45, HHT888-54, No.4(2), No. 4(5), No.5(1), No.5(2), No.5(4), No.5(5), No.5(7), No.5(8), No.5(11) and H and their respective extracts or active principles.

More specifically, there is disclosed a method for reducing the viral load of humans infected with HBV, said method comprising administering to said human a therapeutically effective amount (such as from 0.4 to 120 g per day) of a composition comprising HHT888-5 or an extract obtained from HHT888-5.

There is also disclosed a method for treating humans infected with HCV, said method comprising administering to said human a therapeutically effective amount (such as from 0.4 to 120 g per day) of a composition comprising HHT888-45 or an extract obtained from HHT888-45.

There is also disclosed a method of reducing the viral load of a human carrier of the HBV and a method of treating or preventing hepatitis B in a human, said method comprising administering to said human a therapeutically effective amount (such as from 0.4 to 120 g per day) of at least one composition selected from No.5(5) and at least one agent selected from the group consisting of No.5(1), No.5(2), No.5(3), No.5(4), No.5(6), No.5(7), No.5(8), No.5(9), No.5(10), and No.5(11).

There is further disclosed a method of treating a HCV carrier and a method of treating or preventing hepatitis C in a human, said method comprising administering to said human a therapeutically effective amount (such as from 0.4 to 120 g per day) of a composition comprising the mixture of the single-herb herbal medicine No.5(5), its extract or active principle and at least one single-herb herbal medicine, its extract or active principle selected from the group consisting of No.4(2), No.4(3), No.4(4), No.5(4), No.5(8), and No.5(11).

Also disclosed is a method of treating hepatitis B in a human, said method comprising administering to said human a therapeutically effective amount (such as 0.4 to 120 g per day) of at least one composition selected from HHT888-45 and HHT888-5.

There is further disclosed a method of treating hepatitis B in a human, said method comprising administering to said human a therapeutically effective amount (such as from 0.4 to 120 g per day) of at least one composition selected from: (1) a mixture of the single-herb herbal medicine No.5(5), its extract or active principle and at least one single-herb herbal medicine, its extract or active principle selected from the group consisting of No.4(2), No.4(3), No.4(4), No.5(4), No.5(8), and No.5(11); and (2) a mixture of the single-herb herbal medicine No.5(5), its extract or active principle and at least one single-herb herbal medicine, its extract or active principle selected from the group consisting of No.5(1), No.5 (2), No.5(3), No.5(4), No.5(6), No.5(7), No.5(8), No.5(9), No.5(10), and No.5(11).

There is further disclosed a method for treating humans infected with HIV, said method comprising administering to said human a therapeutically effective amount (such as 0.4 to 120 g per day) of a composition comprising HHT888-4.

There is disclosed a method for treating humans infected with HIV, said method comprising administering to said human a therapeutically effective amount (such as 0.4 to 120 g per day) of a composition comprising HHT888-5.

There is disclosed a method for treating humans infected with HIV, said method comprising administering to said human a therapeutically effective amount (such as 0.4 to 120 g per day) of a composition comprising HHT888-45.

There is disclosed a method for treating humans infected with HIV, HBV and HCV, said method

comprising administering to said human a therapeutically effective amount (such as 0.4 to 120 g per day) of a composition comprising HHT888-54.

There is also disclosed a method for treating humans infected with HIV, said method comprising administering to said human a therapeutically effective amount of a composition comprising at least one single-herb herbal medicine, its extract or active principle selected from the group consisting of No.4(2), No.4(5), No.5(1), No.5(2), No.5(4), No.5(5), No.5(7), No.5(8), No.5(11) and H.

There is also disclosed novel herbal blends and a method of treating humans infected with HIV, said method comprising administering to said human a therapeutically effective amount of an herbal blend comprising at least one herbal medicine selected from No.5(5) H and and mixtures thereof, and at least one herbal medicine selected from the group consisting of No.4(2), No.4(3), No.4(4), No.4(5), No.5(1), No.5(2), No.5(4), No.5(7), No.5(8) and No.5(11).

Also disclosed is a method for treating humans infected with HIV, said method comprising administering to said human a therapeutically effective amount of a mixture comprising at least two antiviral components isolated from the single-herb herbal medicines or their source plants selected from the group consisting of No.4(2), No.4(3), No.4(4), No.4(5), No.5(1), No.5(2), No.5(4), No.5(5), No.5(7), No.5(8), No.5(11), and H.

Also disclosed is a method for treating humans infected with HIV, said method comprising administering to said human a therapeutically effective amount of a composition comprising at least one of the water extractable and acid precipitable antiviral components or compounds isolated from the single-herb herbal medicines or their source plants selected from the group consisting of No.4(2), No.4 (4), No.4(5), No.5(1), No.5(5), No.5(8), No.5(11) and H.

Thus, the present invention is directed to: 1) compositions of matter (i.e., herbal blends and isolated chemical entities); 2) methods for the treatment of HBV and HCV carriers; 3) prevention and treatment of hepatitis B and hepatitis C; 4) treatment of HIV carriers; and 5) prevention and treatment of AIDS through the administration of the compositions according to the present invention.

The dosage of the compositions of the invention can range from 0.4 to 120 g per day for the mammal in need of therapy. One skilled in the art will appreciate that depending upon the weight of the individual and the progression of the viral infection, that higher doses of the compositions may be required. As the compositions according to the invention have demonstrated virtually no side effects, high doses may be initiated with reduction of dosage upon manifestation (i.e., reduction of viral load) of therapeutic effect. One skilled in the art can tailor each dosage rate for a given individual without undue experimentation. More specifically, the dosages for a given composition can range from 0.4 to 100 g per day, more preferably 1.0 to 25 g per day. Preferably, the compositions are administered at least three (3) times per day, however, bolus administration will be effective. More specifically, an oral dosage of 5.5 g three (3) times a day (total 16.5 g per day) of HHT888-5 has been found to be effective to reduce HBV load in carriers. Oral dosage of 2.7-5.7 g three (3) times a day (total 8-17 g per day) of the herb mixture HHT888-45 has been found to be effective to return normal liver function to hepatitis C patients. Dosages as high as 121 g per day for HHT888-5 and 63 g per day for HHT888-45 have not evidenced serious side effects. It should be appreciated that the dosages recited herein are for the herbal medicine (extract deposited on ground plant or adsorbent) in dry form. Further, extracts of the inventive compositions will increase the concentration of the actives and therefore reductions in the dosage levels will be realized. Dosages as low as 10% of those recited herein for the inventive compositions are contemplated. The preferred dosage for No.5(5) to treat HCV infection is from 0.4 to 17 g per day.

The compositions of the invention are preferably administered orally or enterally, however, intravenous

(i.v.) and/or intramuscular (i.m.) administration is also contemplated herein. Those skilled in the art will understand how i.v. and i.m. formulations can be prepared and how the effective dosages can be obtained.

In the method according to this invention a mammal may be a human or animal. The human may be an adult, child or infant. Thus, for infants, an infant formula containing the hereinafter described plant extracts or active principles will be effective in treating the infants infected with HBV, HCV, or HIV. For children and adults, a medical food or nutritional product, such as milks and yogurts, containing the plant extracts or active principles described herein will also be effective in treating humans infected with HBV, HCV, or HIV.

The present invention also relates to a process to isolate the efficacious compounds from the recited herbal medicines or medicinal plants and to the isolated compounds themselves.

The herbs used as starting materials for this invention may be obtained from commercial sources as single-herb herbal medicines which may be mixed, or extracted and concentrated, and placed in compositions for the administration to a human. The plant extracts, once isolated from the plant material, may be concentrated and then placed in form suitable for the administration to a human (i.e., pills, capsules and tablets). The active principles, once isolated from the plant or synthesized, may then be placed in compositions for the administration to a human and may take a variety of forms such as capsules, tablets, powder, candies, gels, beverages, teas, nutritional products, and the like.

Also disclosed is a medicinal product produced by the process comprising the steps of: (a) contacting comminuted plant material such as No.5(1) to No.5(11), No.4(2) to No.4(5), H, and mixtures thereof, with water to form an aqueous dispersion; (b) heating the aqueous dispersion to about 100.degree. C. and holding at that temperature for about 0.5 to about 3 hours; (c) separating the insoluble plant material from the aqueous phase; and (d) concentrating the solute contained in the aqueous phase. The concentrated solute may be obtained through freeze drying, spray drying, evaporation or ultrafiltration.

Also disclosed is a medicinal product produced by the process comprising the steps of: (a) contacting comminuted plant material selected from the group consisting of No.4(2), No.4(4), No.4(5), No.5(1), No.5(5), No.5(8), No.5(11), H, and mixtures thereof, with water to form an aqueous dispersion; (b) heating the aqueous dispersion to about 100.degree. C. and holding at that temperature for about 0.5 to about 3 hours; (c) separating the insoluble plant material from the aqueous phase; (d) acidifying the aqueous solution with acid (such as hydrochloric acid) to a pH of less than about 2; (e) separating the acid precipitate from the supernate; and (f) purifying the acid precipitate by dissolving in a neutral or basic solution (such as 0.1 N ammonium bicarbonate) and precipitating again with acid. Optionally, the acid precipitate may be dissolved in 0.1 N ammonium bicarbonate solution and concentrated. The concentrated solute may be obtained through freeze drying, spray drying, evaporation or ultrafiltration.

Representative of the acids that are useful in acidifying the aqueous extracts include hydrochloric acid, phosphoric acid, glacial acetic acid, sulfuric acid and the like. What is important is that the acid have a pKa sufficient to convert the active components to the acid form. The pH of the extract should be less than 3 and most preferably less than 2 for precipitation to occur.

Also disclosed is a medicinal product produced by a process comprising the steps of: (a) contacting at least one herbal medicine selected from: No.4(2), No.4(4), No.4(5), No.5(1), No.5(5), No.5(8), No.5(11), H, and mixtures thereof, with water to form an aqueous dispersion; (b) stirring the aqueous dispersion at ambient temperature for about 0.5 hours or longer, to about 3 preferably 0.5 hours; (c) separating the insoluble plant material from the aqueous phase; (d) acidifying the aqueous solution with acid to approximately a pH of less than 2 to form a precipitate; (e) separating the acid precipitate from

the acid supernate; and (f) purifying the acid precipitate. The precipitate may be purified by repetitively dissolving it in 0.1 N ammonium bicarbonate solution and precipitating it again with acid.

This application sets forth the data available on the present discoveries and fully describes the compositions of matter, their preparations, clinical applications, and analytical tools used to characterize the various active components. These and other aspects of the invention will become apparent to those skilled in the art as a result of the following examples which are intended as illustrative of the invention and not limitative.

BEST MODE FOR CARRYING OUT THE INVENTION

To acquaint persons skilled in the art with the principles of the invention, the following Examples are submitted which are intended to be illustrative and not limitative. All percentages are percentages by weight unless otherwise specified.

EXAMPLE 1

Preparation of Herb Mixtures

In the preparation of the herbal compositions according to the invention, Chinese herbal medicines in single herb format were obtained from commercial sources in powder form. The individual single-herb herbal medicines were mixed in the appropriate proportions to prepare each herb mixture.

The herb mixture for HHT888-4 was prepared by mixing No.4(1), No.4(2), No.4(3), No.4(4), and No.4(5) at a ratio of 3:3:3:3:4 by weight. The herb mixture HHT888-5 was prepared by mixing equal weights of No.5(1), No.5(2), No.5(3), No.5(4), No.5(5), No.5(6), No.5(7), No.5(8), No.5(9), No.5(10), and No.5(11).

The herb mixture HHT888-45 was prepared by mixing four (4) to six (6) single-herb herbal medicines No.4(3), No.4(4), No.5(4), No.5(5), No.5(8), and No.4(2) at a ratio of 1:1:1:1:0-1:0-1 by weight. The single-herb herbal medicine No.5(8) or No.4(2), or both, were not used in some cases in HHT888-45 for initial administrations. One of the two single-herb herbal medicines or both were added later when needed to enhance the therapy. The weight ratio of the single-herb herbal medicine No.4(2) in the herb mixture HHT888-45 also varied case-by-case between 0.5 and 1 when used.

It is noted that a mixture of decoctions prepared individually from the source plants of the single-herb herbal medicines or a decoction prepared from the pre-mixed source plants of the single-herb components of each herb mixture is within the scope of this invention.

EXAMPLE 2

Preparation of Single-herb Herbal Medicines

The plant source from which each single-herb herbal medicine was obtained is listed in the Prior Art and Summary sections of this application. It should be understood that more than one species or genus of medicinal plant may be used to prepare the same herbal medicine. For example, the herbal medicine No.5(8) or FORSYTHIAE FRUCTUS may be prepared from three (3) species of Forsythia genus plants, i.e., Forsythia suspensa, Forsythia viridissima, Forsythia koreana or mixtures thereof. The herbal medicine No.5(6) (BAPHICACANTHIS RHIZOMA ET RADIX) may be prepared from one of the five (5) plants Baphicacanthes cusia, Strobilanthes cusia, Isatis tinctoria, Isatis indigotica, Polygonum tinctorium or mixtures thereof. The herbal medicines were prepared from their respective plant sources

as follows.

A suitable part or parts or the whole plant was obtained, washed with cold water, dried and comminuted. The plant materials were then extracted with boiling water on a basis of 1 part by weight of plant material to approximately 5 to 10 parts by weight of water. The amount of water used should at least cover the plant material in the extraction vessel. Samples were boiled for 0.5 to one hour, but not in excess of 3 hours, in order to allow effective extraction of the desired components. Shorter or longer heating would not substantially affect the extraction, except the yield and cost. The aqueous solution was separated from the plant material by filtration.

The aqueous solution may be freeze dried or spray dried, or reduced in volume by heating with or without an applied vacuum. The concentrate may then be spray dried or freeze dried or absorbed onto a powdered form of the same plant material, starch or other absorbant. Thus the single-herb herbal medicine is prepared.

A decoction is the aqueous solution of the plant material prepared by boiling the plant material in water as described above for about 0.5 to one hour. The decoction may be directly consumed after it is prepared and cooled to warm or ambient temperature or preserved with proper sterilization for later consumption. Sterilization may be accomplished by microfiltration or heat.

EXAMPLE 3

Treatment of Hepatitis B Virus Carriers

Twenty-nine (29) HBV carriers with normal levels of serum glutamine oxalacetate transferase (SGOT) and glutamine pyruvate transferase (SGPT) (liver enzymes), were treated with HHT888-5. Several HBV carriers who had elevated SGOT and SGPT levels were first treated with other remedies which returned their serum liver enzymes to normal levels (8-40 unit/mL for SGOT and 5-35 unit/mL for SGPT) but failed to reduce the HBV load. Treatment with HHT888-5 then began. HHT888-5 was prepared as described in Example 1 by mixing eleven (11) single-herb herbal medicines which were obtained from a commercial source and were manufactured following good manufacture practice (GMP) guidelines. Consent of each patient was obtained before their treatment began.

Patients were instructed to take the HHT888-5 three (3) times a day. Each dose was 5.5 g. Each 5.5 g packet of the herb mixture was mixed with warm water and consumed orally. Serum hepatitis B surface antigen (HBsAg) titers of each patient were determined at intervals as shown in Table 1 to monitor the progress of the treatment. Serum HBsAg titer was determined using a reverse-passive hemagglutination test as described in: (1) Instruction of "Taifu" Serodia-HBs Test Reagent for HBsAg Detection, Taifu Pharmaceutical Co., Ltd., Taoyuan, Taiwan, R.O.C.; (2) D. S. Chen & J. L. Sung, J. Formosan Med Assoc., 77, 263-270 (1978); and (3) T. Juji & T. Yokochi, Japan. J. Exp. Med., 39, 615-620 (1969).

Table 1 shows the treatment results of the twenty-nine (29) HBV carriers. Patients showed improvement in their disease state over the course of treatment, as indicated by their HBsAg titer reductions and well being. Fourteen (14) carriers (48%) whose HBsAg titers ranged from 20 to 81,920 were significantly lowered (four to 256-fold reductions, or from positive to negative) after 35 to 964 days of treatment. Four (4) carriers (14%) reduced their HBsAg titers from 20, 40, and 2,560 to negative (i.e., below 20 ng/mL detection level) after 56-153 days of treatment. Fourteen (14) carriers (48%) had no significant change (two-fold titer decrease or increase or no change) in HBsAg titers during the course of the treatment (63-284 days). One carrier (3%) had a slightly four-fold titer increase.

TABLE 1

Clinical Effects of HHT888-5 on Hepatitis B Virus Carriers

PATIENT	HBsAG Titer BEFORE	AFTER	DURATION (Days)
1	40	negative	
			56
2	2560	negative	
			72
3	20	negative	
			153
4	20	negative	
			88
5	2560	80	53
6	1280	320	101
7	2560	1280	32
		1280	399
		320	964
8	2560	1280	79
		640	412
9	20480	5120	53
10	20480	5120	60
11	40960	10240	35
12	81920	40960	74
		10240	461
13	81920	20480	63
14	5120	2560	170
		2560	245
		1280	556
		1280	832
15	160	80	284
16	320	160	198
17	640	320	276
18	1280	640	120
19	2560	1280	69
20	5120	2560	263
21	20480	10240	77
22	40960	40960	120
		20480	210
23	160	160	227
24	320	320	79
25	640	640	157
26	1280	1280	69
27	40960	40960	137
28	5120	10240	63
29	160	640	121

The HHT888-5 treatment set forth in this Example compares very favorably with the currently accepted interferon therapy. The response rates for interferon therapy and HHT888-5 treatment to lower the HBsAg titers in patients infected with HBV are comparable, approximately 40% vs. 48%, respectively. The serum HBsAg clearance rates were also comparable for both, 10 to 15% for interferon therapy and approximately 14% for HHT888-5 treatment. Furthermore, the interferon therapy is typically administered intramuscularly or intravenously, with frequent adverse effects. The HHT888-5 treatment was administered orally (like drinking a tea) with no apparent side effects in all patients treated. Oral administration is much more convenient and more economical than intramuscular or intravenous

administration. HHT888-5 can thus be safely and conveniently consumed even on a long-term basis to reduce or control HBV proliferation in HBV carriers and hepatitis B patients.

When the HBV viral load in an HBV carrier can be reduced or maintained at a sufficiently low level, the carrier is much less likely to progress to hepatitis, liver cirrhosis, liver cancer, and death. Thus, HHT888-5 can be used to prevent and treat hepatitis B, or even prevent liver cirrhosis or liver cancer caused by HBV infection.

Since HHT888-5 was administered in this Example by mixing the powder in water first and then consumed orally, isolation of the active components of HHT888-5 and its administration to humans would also be efficacious in the treatment of HBV. Dosages of the herb mixture HHT888-5 as high as 120 g per day have been accomplished without serious side effects.

EXAMPLE 4

Antiretroviral Testing of Herb Mixtures and their Water Extracts

In this example, two herb mixtures, HHT888-4 and HHT888-5, were tested for their antiretroviral activities and found to be active against EMuLV and HIV in an in vitro assay. Two in vitro assays, anti-Ecotropic Murine Leukemia Virus (anti-EMuLV) and anti-HIV, were used to test the antiretroviral activities of the inventive compositions.

The anti-EMuLV assay uses a large, enveloped, RNA-containing retrovirus, EMuLV, which belongs to the same virus family as HIV and has many characteristics that are similar to HIV.

1. Anti-Ecotropic Murine Leukemia Virus Assay

The assay contained two parts, a cytotoxicity test and a virus suppression test. See QBI Protocol 39014 Final Report and QBI Protocol 39016 Final Report, Quality Biotech, Camden, N.J., USA, 1992. Each sample was initially tested for its cytotoxicity to the SC-1 indicator cells which were used for titration of infectious EMuLV in a XC plague assay. Cytotoxicity as reported herein is expressed in terms of percent of control proliferation. The higher the percent means the substance being tested is not toxic to the cells. This is very important as compounds that are highly toxic would scew the interpretation of the assay results. For example, high activity in an HIV assay and a high cytotoxicity (low % of control proliferation) could mean that the test compound is inhibiting the growth of the host cells thereby limiting the growth of the virus. Thus, a false positive on anti-viral activity could be interpreted. See QBI protocol C30015, Quality Biotech, Camden, N.J., USA. Each sample was dispersed in a virus resuspension buffer (50 mM Tris, pH 7.8, 10 mM KCL, 0.1 mM EDTA) without the virus. The solution was then subjected to the XC plague assay under the same conditions as those for the determination of EMuLV titer. A sample was considered cytotoxic if the indicator cells for the assay were less than 50% confluent. A noncytotoxic sample concentration was chosen for the virus suppression test.

In the virus suppression test, each sample was incubated with EMuLV (strain AKV623, titer 2.2-4.2.times.10.sup.5 PFU/mL) in a virus resuspension buffer at 23-25 mg/mL (e.g., 100 mg/4.0 mL) for 12-32 minutes. The treated virus suspension was pH adjusted, if necessary, to within 6.8-7.2 and then tested for its titer in the XC plague assay.

An aliquot (1.5 mL) was diluted in the cell culture medium to the endpoint (10.sup.0, 10.sup.-1, 10.sup.-2, 10.sup.-3,10.sup.-4, 10.sup.-5,10.sup.-6, 10.sup.-7, and 10.sup.-8 dilutions, or as appropriate). Each dilution was vortexed to resuspend any particulates if present and assayed in duplicate for infectious viral particles by the XC plaque assay. A positive control (virus suspension without treatment) and a

negative control (cell culture medium, no virus) were also analyzed concurrently to validate the assay.

Anti-EMuLV activity of the sample was expressed in log.sub.10 reduction of the EMuLV titer when compared to the positive control. A sample with log.sub.10 titer reduction greater than 0.5 is considered to be active.

HHT888-4 and HHT888-5 were initially tested "as is" and exhibited good antiviral activities (1.0 to 1.4 log.sub.10 reduction in viral titer) at 25 mg/mL and 12 minutes of incubation with the virus at room temperature. They were then tested again with a longer incubation time (32 minutes) with the virus at the same concentration. Each sample was also tested for its soluble and insoluble fractions in the above virus resuspension buffer to see if any active component was water soluble. The soluble portion was separated from the insoluble one by centrifuge at room temperature and 10,000.times.g for 10 minutes. The soluble fraction was divided into two aliquots, one 0.45-.mu.m filtered and one unfiltered, and tested to see if residual particulates have any effect on the activity.

Table 2 summarizes the anti-EMuLV activity test results. The results confirmed that both HHT888-4 and HHT888-5 and their soluble and insoluble fractions have anti-EMuLV activities. The samples caused 1.0 to 2.6 log.sub.10 reduction in viral titer when they were incubated with the virus at 23-25 mg/mL for 32 minutes. Microfiltration did not significantly affect the activity of either soluble fraction.

TABLE 2

Anti-Ecotropic Murine Leukemia Virus Activity

Sample	Treatment	Cytotoxicity* 25	2.5	0.25 mg/mL	Anti-EMuLV Activity Log.sub.10 Titer Reduction**
HHT888-4					
	"as is"	Yes	No	No	1.02 (90%)***
	"as is"	Yes	No	No	1.04 (91%)****
	Soluble	--	--	--	1.74 (98%)****
	Soluble, filtered	--	--	--	1.59 (97%)****
	Insoluble	--	--	--	2.64 (99.8%)****
HHT888-5					
	"as is"	Yes	No	No	1.35 (96%)***
	"as is"	Yes	No	No	2.10 (99.2%)****
	Soluble	--	--	--	2.05 (99.1%)****
	Soluble, filtered	--	--	--	1.71 (98.1%)****
	Insoluble	--	--	--	1.72 (98.1%)****

*Sample was considered cytotoxic if the SC1 indicator cells for the assay were less than 50% confluent.

**As compared to a working virus suspension with a titer of 2.2-4.2 .times. 10.sup.5 PFU/mL (plaque forming units/ml), or Log.sub.10 (PFU/mL) = 5.34-5.62. The values in parentheses indicate percent reductions in viral titer from the working virus suspension.

***Incubation time 12 minutes, at 25 mg/mL test level. The activity may b caused by the sample, by microbial contaminant, or by a nonspecific physical interaction between the particles of the sample and the virus, since the samples were not sterile filtered before assay.

****Incubation time 32 minutes, at 25 mg/mL test level for the "as is"

unfractionated samples. For soluble, soluble & sterile filtered, and insoluble fractions, the test level was equivalent to 23 mg/mL of its unfractionated sample.

The data contained in Table 2 demonstrates that HHT888-4 and HHT888-5 are effective anti-EMuLV agents.

2. Anti-Human Immunodeficiency Virus Assay

This assay also contained two parts, a toxicity test and an HIV suppression test. The sample was mixed in a cell culture medium, e.g., 50 mg in 1.00 mL. The mixture was vortexed and centrifuged to separate the soluble from the insoluble. The supernate was filtered through a 0.45-.mu.m filter and then diluted with cell culture medium to appropriate concentrations for the assay. The cell culture medium used in the assay was RPMI 1640 (pH 7.3.+-.0.3) supplemented with 10% fetal calf serum, 2 mM glutamine, 50 U/mL penicillin and 50 .mu.g/mL streptomycin.

The sample was tested for its cytotoxicity and/or cytostatic activity towards the target cells, human peripheral blood lymphocytes (PBLs). A lymphocyte proliferation assay was used for the toxicity test, where a 100 .mu.L sample was incubated with 100 .mu.L of a cell suspension of uninfected PBLs (3.times.10.sup.5 cells) under the same conditions as the HIV suppression test. Lymphocyte proliferation was measured by a colorimetric assay (MTT-Test). See T. Mosmann, J. Immunological Methods, 65, 55-63 (1983). A sample concentration which results in .gtoreq.70% of the control in lymphocyte proliferation is considered to be acceptable for the HIV suppression test.

In the HIV suppression test, HIV-1 infected PBLs were cultivated in the presence of the sample for four (4) days as in the toxicity test. See H. Ruebsamen-Waigmann, et al., J. Med Virology, 19, 335-344 (1986). The secreted viral core protein p24 and/or viral RNA were determined as indicators for virus proliferation status on day 3 and day 4 by an HIV-1 p24 capture ELISA technique and an HIV-RNA dot blot hybridization technique, respectively. The concentration of p24 synthesized by the HIV infected cells was determined by Sandwich ELISA. A standard preparation of recombinant p24 (MicroGeneSys, USA) was used for calibration of the ELISA. See Ch. Mueller, et al., Fresenius Z. Anal. Chem., 330, 352-353 (1988).

HIV-RNA synthesized in the infected cells was determined by a nucleic acid hybridization technique. Cellular RNA was prepared from the infected cells and analyzed by a dot blot hybridization technique. The hybridization solution contained the P.sup.32 -labeled DNA probe which comprised a 5.5 kilobase DNA fragment of the HIV isolate D.sub.31. See H. v. Briesen, et al., J. Med. Virology, 23, 51-66 (1987). This fragment covering the gag/pol region of the virus is labeled with P.sup.32 alpha-d CTP by oligonucleotide labeling. Plus-strand RNA transcripts derived from the gag/pol region of the viral isolate D.sub.31 were used as the external standard for the hybridization. These "run-off" transcripts were generated by means of the T7 polymerase reaction from negatively polarized HIV-DNA under T7-promotor control. The concentration of RNA transcripts was determined spectrophotometrically. The hybridized probe was detected by autoradiography and the processed autoradiograms were evaluated densitometrically.

A positive control, a negative control, and an AZT control were conducted concurrently to assure the validity of the HIV suppression test. All tests were performed in triplicates, and 96-well round bottom microtiter plates were used for all assays.

A positive control was HIV-1 infected lymphocytes cultivated in the presence of the cell culture medium without the sample. A negative control was lymphocytes infected with a heat-inactivated virus inoculum incapable of replication. These "mockinfected" lymphocytes were cultivated and assayed in the same way as the infected cells. The amount of viral protein being present in the cultures solely due to the remaining inoculum was thus determined as the background level. The amount of viral protein p24 in the test sample and in the positive control due to viral replication was then determined by the respective p24 levels less the background level.

The amount of viral protein being present in the cultures containing the sample due to viral proliferation was compared with that in the positive control, i.e., the culture without the sample. The % suppression of HIV proliferation was determined by the difference in p24 levels between the positive control and the sample, divided by the p24 level of the positive control, and timed 100%.

The AZT control was conducted via HIV-1 infected lymphocytes that were cultivated in the presence of azidothymidine (AZT) at concentrations of 100, 10, 1 and 0.1 ng/mL, respectively. This provided an estimate of the sensitivity of the lymphocytes towards AZT, a known inhibitor of HIV-1 replication. The suppression of HIV-1 proliferation caused by AZT in a concentration of 10 ng/mL should be greater than 50% as compared to the untreated positive control.

Table 3 summarizes the cytotoxicity and the HIV suppression test results of HHT888-4 and HHT888-5, as well as the AZT controls. Both herb mixtures were active in suppressing HIV proliferation in infected human lymphocytes at 2.5-5.0 mg/mL, but not at 50 .mu.g/mL (50-100 times diluted). The AZT controls from all sets exhibited the expected activities and thus assured the validity of the tests.

TABLE 3

Anti-HIV Activities of HHT888-4 and HHT888-5

Test Sample	Concentration	Cytotoxicity*	HIV Suppression p24		RNA	
			Day 3	Day 4	Day 3	Day 4
HHT888-4						
	2.5 mg/mL					
		>46%	100%	100%	100%	100%
	50 g/mL					
		85%	1%	6%	--	--
HHT888-5						
	5.0 mg/mL					
		75%	100%	97%	99%	100%
	50 g/mL					
		86%	0%	12%	--	--
AZT	100 ng/mL					
		--	99-100%	100%	--	--
	10 ng/mL					
		--	85-98%			

			77-96%		
				--	--
1 ng/mL					
	--	20-39%			
			8-12%		
				--	--
0.1 ng/mL					
	--	0%	0-3%		
				--	--

*Percent proliferation of control. HHT8884 was 46% at 5.0 mg/mL. Both HHT8884 and HHT8885 were cytotoxic (<50% of control) at 25 mg/mL level.

At 2.5-5.0 mg/mL of HHT888 to 4 and HHT888 to 5, HIV proliferation in infected human lymphocytes was essentially completely suppressed: 97 to 100% suppression based on viral protein p24 and 99 to 100% suppression based on viral RNA determined on both day 3 and day 4 after treatment. The anti-HIV activity at 50 .mu.g/mL was negligible, 0 to 12% suppression for both herb mixtures. The activities could not be attributed to insoluble particulates since they were filtered out by a 0.45-.mu.m filter before the assay and the activities were not due to cytotoxicity. Repeat tests on three lots of HHT888-4 showed 100% suppression at 2.5 mg/mL on both day 3 and day 4 with acceptable cytotoxicity (71 to 100% of control proliferation). Repeat tests on three lots of HHT888 to 5 at 2.5 mg/mL showed 93-98% suppression on day 3 and 89 to 99% suppression on day 4 with acceptable cytotoxicity (85 to 91% of control proliferation). Results of the repeat experiments are shown in Table 4.

TABLE 4

Anti-HIV Activities of HHT888-4 and HHT888-5 and their Water Extracts

Sample	Lot	% Weight	Test Concentration	HIV Suppression** Cytotoxicity*	Day 3	Day 4
HHT888-4						
	1	100%	2.5 mg/mL	>46%	100%	100%
			2.5 mg/mL	98%	100%	100%
			0.05 mg/mL	85%	1%	6%
	2	100%	2.5 mg/mL	100%	100%	100%
	3***	100%	2.5 mg/mL	71-79%	100%	100%
HHT888-4-E1						
	2	17%	1.0 mg/mL	98%	100%	96%
E2	2	11%	1.0 mg/mL			

				96%	100%	
						87%
E	2	28%	1.0 mg/mL			
				47%	100%	
						100%
			0.5 mg/mL			
				78%	100%	
						100%
	4	27 .+-. 1%****				
			1.0 mg/mL			
				72%	100%	
						100%
			1.0 mg/mL			
				100%	100%	
						93%
			0.1 mg/mL			
				97%	34%	12%
			0.02 mg/mL			
				82%	23%	2%
HHT888-5						
	1	100%	5.0 mg/mL			
				75%	100%	
						97%
			2.5 mg/mL			
				89%	93%	91%
			0.05 mg/mL			
				86%	0%	12%
	2	100%	2.5 mg/mL			
				91%	94%	89%
	3***					
		100%	2.5 mg/mL			
				44-85%	98%	99%
			0.5 mg/mL			
				52-100%		
					0%	0%
HHT888-5-E						
	2	19%	1.0 mg/mL			
				91%	71%	26%

*Toxicity in percent of control proliferation.
**HIV suppression based on viral protein p24 levels.
***Composite of respective single herb components at equal proportions. No.5(10) and No.5(11) were not included in Lot 3 of HHT8885.
****Based on two (2) runs.

It is noted that Lot 3 of HHT888-4 or HHT888-5 was prepared by mixing the respective single-herb components at equal proportion by weight. Lot 3 of HHT888-5 was composed of nine (9) single-herb components, excluding No.5(10) and No.5(11).

Water extracts of HHT888-4 and HHT888-5 (E to E2) from one to two lots were further tested to see whether the active components were extractable by water. Water extracts of HHT888-4 and 5 were prepared by extracting 5 g of the powder with 25 mL of MilliQ purified water twice. Each water suspension was vortexed for 1 minute, stood for 5 minutes, and vortexed again for 1 minute to facilitate the extraction. The extract was separated from the insoluble by centrifuge at 1,000-2,000 rpm for 20 minutes. The supernate was transferred into a clean preweighed 50-mL centrifuge tube, freeze dried,

weighed, and tested for anti-HIV activity.

The percent weight of material extracted was 17.3% for the first 25 mL extract and 10.8% for the second 25 mL extract of HHT888-4 (Lot 2). That was 14.2% for the first 25 mL extract and 4.6% for the second 25 mL extract of HHT888-5 (Lot 2). The first (E1), the second (E2) and the combined (E) extracts of HHT888-4 (Lot 2) were tested for anti-HIV activity. All the other extracts were tested with the first and the second extracts combined. The results are also set forth in Table 4.

All three lots of each of the herb mixtures were very active, 100% suppression at 2.5 mg/mL for HHT888 to 4 and 89-100% suppression at 2.5 to 5.0 mg/mL for HHT888 to 5. The IC.sub.50 was between 0.05 to 2.5 mg/mL for HHT888 to 4 and between 0.5 to 2.5 mg/mL for HHT888 to 5. IC.sub.50 is the concentration of the test substance at which would cause 50% suppression of the viral proliferation.

The water extract of HHT888-4 showed very good activity: 93 to 100% suppression at 0.5 to 1.0 mg/mL. The first (E1) and the second water extract (E2) of Lot 2 exhibited comparable activities: 100% suppression on day 3 and 87 to 96% suppression on day 4 at 1.0 mg/mL. The IC.sub.50 of the water extract of HHT888 to 4 was between 0.1-0.5 mg/mL.

The water extract of HHT888-5 (Lot 2) exhibited a substantially lower activity: 71% suppression on day 3 which dropped to 26% suppression on day 4 at 1.0 mg/mL. The main active component apparently stayed behind in the insoluble fraction and was not as easily extracted by water as that of HHT888-4 under the aforementioned conditions. It is noted that the water extract of HHT888-5 (Lot 2) constituted 19% by weight of the herb mixture. The test concentration of the water extract of HHT888-5 (or HHT888-5-E) at 1.0 mg/mL is equivalent to 5.3 mg/mL of HHT888-5 itself. HHT888-5 was tested very active at both 2.5 mg/mL (93 to 98% suppression on day 3 and 89 to 99% on day 4) and 5.0 mg/mL (100% suppression on day 3 and 97% on day 4).

The above results clearly demonstrated that both HHT888-4 and HHT888-5 and their water extracts have in vitro antiretroviral activities, more specifically anti-EMuLV and anti-HIV activities. HHT888-5 has also been shown to be efficacious in treating hepatitis B virus carriers.

EXAMPLE 5

Antiretroviral Testing of Individual Single-herb Herbal Medicines

In this experiment, the individual single-herb components of HHT888-4 and HHT888-5 were tested for anti-HIV activity. Table 5 sets forth the test results.

TABLE 5

Anti-HIV Activities of Single-herb Components of HHT888-4 and HHT888-5

Sample	Lot	Test Concentration	Cytotoxicity*	HIV Suppression** Day 3	Day 4
No. 4(1)***	1	2.5 mg/mL	98%	73%	50%
No. 4(2)	1	2.5 mg/mL	74-84%	92%	94%
No. 4(3)					

1	2.5 mg/mL	75-78%	100%	100%
No. 4(4)				
1	2.5 mg/mL	74-100%	100%	100%
No. 4(5)				
1	2.5 mg/mL	41-79%	98%	92%
	0.5 mg/mL	47-100%	0%	0%
No. 5(1)***				
1	2.5 mg/mL	98%	73%	50%
No. 5(2)				
1	2.5 mg/mL	73-87%	18%	29%
No. 5(3)				
1	2.5 mg/mL	89-100%	0%	0%
No. 5(4)				
1	2.5 mg/mL	64%	100%	100%
	1.0 mg/mL	69-91%	0%	0%
No. 5(5)				
1	2.5 mg/mL	80-84%	93%	93%
No. 5(6)				
1	2.5 mg/mL	94-100%	0%	0%
No. 5(7)				
1	2.5 mg/mL	90-100%	50%	38%
No. 5(8)				
1	2.5 mg/mL	32-59%	100%	100%
	0.5 mg/mL	65-100%	0%	0%
No. 5(9)				
1	0.5 mg/mL	24-78%	0%	0%
No. 5(10)				
1	2.5 mg/mL	100%	65%	0%
No. 5(11)				
1	2.5 mg/mL	100%	92%	74%

*Toxicity in percent of control proliferation.
**HIV suppression based on viral protein p24 levels.
***No. 4(1) = No. 5(1)

All five (5) single-herb components of HHT888-4 exhibited anti-HIV activities with various degrees: 73 to 100% suppression on day 3 and 50 to 100% suppression on day 4 at 2.5 mg/mL. No.4(3) and No.4(4) exhibited the best activity: 100% suppression at 2.5 mg/mL on both day 3 and day 4. No.4(2) and No.4 (5) were the next: 92 to 98% suppression on day 3 and 92 to 94% suppression on day 4 at 2.5 mg/mL. No.4(1) exhibited a moderate activity: 73% suppression on day 3 and 50% suppression on day 4 at 2.5 mg/mL. No.4(5) exhibited a slight cytotoxicity (41 to 79% of control proliferation) which was likely to contribute to the observed activity with an ID.sub.50 between 0.5 and 2.5 mg/mL.

Three (3) of the eleven (11) single-herb components of HHT888-5: No.5(4), No.5(5), and No.5(8) exhibited very good activities, 93 to 100% suppression of HIV proliferation on both day 3 and day 4 at 2.5 mg/mL. No.5(11) was the next: 92% suppression on day 3 and 74% suppression on day 4 at 2.5 mg/mL. Again, No.5(1), which was the same as No.4(1), had a moderate activity: 73% suppression on day 3 and 50% suppression on day 4 at 2.5 mg/mL. No.5(2) and No.5(7) exhibited only marginal activities: 18 to 50% suppression on day 3 and 29-38% suppression on day 4 at 2.5 mg/mL. No.5(10) exhibited a very slight activity: 65% suppression on day 3 which dropped to 0% on day 4 at 2.5 mg/mL. The remaining three (3) single-herb components, No.5(3), No.5(6), and No.5(9) exhibited no activity at 0.5 to 2.5 mg/mL. No.5(9) was not tested at 2.5 mg/mL level because of its cytotoxicity: already 24-78% of control proliferation at 0.5 mg/mL.

Although No.5(4) and No.5(8) appeared to be slightly more active than No.5(5) (100% vs. 93% suppression at 2.5 mg/mL), their activities might be partially due to cytotoxicity (32 to 64% of control proliferation at 2.5 mg/mL). This was supported by the loss of activity (0% suppression) when tested at lower levels, 0.5 to 1.0 mg/mL, where the cytotoxicity was lower and more acceptable to the assay.

EXAMPLE 6

Anti-HIV Testing of Medicinal Plant

The source plant of the single-herb herbal medicine No.5(5), Aeginetia indica, was obtained from a local herbal store in Taiwan and tested for its anti-HIV activity. This was to see whether the activity can be reproduced in the herbal medicine prepared directly from its source plant, instead of being obtained from the commercial source.

The whole plant was washed with cold water, dried, comminuted, and extracted with boiling water as described in Example 2. The aqueous solution was separated from the plant material by filtration. The aqueous solution was then reduced in volume by heating. The concentrate was spray dried and absorbed onto powdered material of the same plant material and thus was prepared the herbal medicine in powder form, designated hereinafter as raw No.5(5).

The powdered herbal medicine prepared from Aeginetia indica, or raw No.5(5), was extracted with water at ambient temperature. Two (2) 5.00 g samples were each extracted twice with about 40 mL of water each time in a separate 50-mL plastic centrifuge tube by vortexing for one (1) minute, standing for ten (10) minutes, and vortexing again for one (1) minute. The tubes were centrifuged at 1500 rpm for twenty (20) minutes to separate the extracts from the insoluble residues. The extracts were filtered through a Whatman No.4 filter paper, freeze dried or nitrogen dried, and weighed.

The above extraction of the raw No.5(5) with water (pH .about.5.1) was repeated and the pH of the first extract was measured to be 5.7. The first and the second extracts were respectively separated from the residue, air dried, and weighed. The percent weight of the extractable was determined to be 18.7.+-.2.8% (n=2).

The first water extract of the raw No.5(5) was tested for anti-HIV activity and found to be active, 91% suppression on day 3 and 97% suppression on day 4 at 1.0 mg/mL. Cytotoxicity test showed that the extract was not cytotoxic at this level, 99% of control proliferation.

EXAMPLE 7

Treatment of Hepatitis C Patients

HHT888-5 has been demonstrated to be effective and safe in treating HBV infections in humans. That means, the active principle or principles of HHT888-5 must be bioavailable in humans through oral administration to cause the decrease of HBV in those patients treated, as indicated by the decrease of their HBV surface antigen (HBsAg) exhibited in Example 3. In addition, Hozumi et al. provided examples in U.S. Pat. No. 5,411,733 to support the belief that substances exhibiting antiviral activity in vitro also possess antiviral activity in vivo as described in the Prior Art section. It is therefore logical to believe that HHT888-4 or HHT888-5 and their water extracts or active principles should also be effective for treating HIV infections in humans.

To test this belief, six (6) of the most anti-HIV active single-herb components of HHT888-4 and HHT888-5 were selected to treat hepatitis C patients caused by HCV infections. The logic is that both

HCV and HIV are retroviruses. Viral hepatitis C tends to become a chronic disease and is therefore more suitable for the test of the treatment. If the treatment works for patients infected with HCV, it will also work for patients infected with HIV. Example 7 clearly demonstrates the validity of this belief.

Six (6) of the most anti-HIV active single-herb components of HHT888-4 and HHT888-5 were selected and mixed to treat hepatitis C patients caused by HCV infections. The six (6) single-herb herbal medicines selected were No.4(2), No.4(3), No.4(4), No.5(4), No.5(5), and No.5(8). No.4(5) was not included although it exhibited a very good activity, because it was learned that the herb might have a certain unconfirmed toxicity.

The six (6) single-herb herbal medicines were obtained from a commercial source and were manufactured following good manufacture practice (GMP) guidelines. They were mixed according to the desired ratio in various combinations and thus the herb mixture HHT888-45 was prepared as described in Example 1. Patients' consents were obtained before the initiation of treatment.

Patients were instructed to take the herb mixture three (3) times a day, 2.7 to 5.7 g each time. Unit dosages of the herb mixture HHT888-45 were prepared in individual packets. Each unit dose packet (2.7 to 5.7 g) of the herb mixture was mixed with warm water and taken orally. All patients were treated with HHT888-45 containing No.4(3), No.4(4), No.5(4), and No.5(5). No.5(8) or No.4(2) or both were added in HHT888-45 for the treatment of some patients at the very beginning or during the course of the treatment to enhance the effectiveness of the treatment.

During the course of the treatment, the daily dose of No.4(3), No.4(4), No.5(4), and No.5(5) varied from two (2) to three (3) grams each. The daily dose of No.5(8) also varied from two (2) to three (3) grams when used. The daily dose of No.4(2) varied from 1.5 to two (2) grams when used. The dose was varied according to the progress of the disease.

Seven (7) viral hepatitis C patients were treated. Their serum liver enzymes, SGOT and SGPT, were determined from time to time by a local clinical laboratory during the course of the treatment to monitor the progress of the disease. The SGOT and SGPT were determined using an enzyme assay. See (1) Instruction of Kyokuto TA-E Transaminase Assay Reagents, Permit No. (62AM)0885, Kyokuto Pharmaceutical Industry Co., Ltd., Tokyo, Japan, 1994; (2) Instruction of Yatrozyme TA-Lq Transaminase-assay Reagent Solution (Enzyme Assay), Commodity No. 817245 (RM163-K), Yatron Co., Ltd., Diayatron Co., Ltd., Tokyo, Japan; and (3) U. Lippi & G Guidi, Clin. Chem. Acta., 28, 431-437 (1970).

The levels of serum GOT and GPT closely correlate with the degree of cellular injury in the liver. These tests are widely used in the diagnosis of liver diseases and as an indicator of the liver function. The normal range for SGOT is 8-40 units/mL and that for SGPT is 5-35 units/mL. Elevated SGOT and SGPT levels usually indicate compromised liver functions.

The results of HHT888-45 treatment are shown in Table 6. All seven (7) patients treated had their serum liver enzymes returned from elevated levels (SGOT from 48 to 166 unit/mL and SGPT from 41 to 291 unit/mL) to essentially normal range (SGOT from 8 to 40 unit/mL and SGPT from 5 to 35 unit/mL) after 17 to 178 days of treatment. Thus, the liver functions of the patients were returned to normal after consumption of the inventive composition.

TABLE 6

Clinical Effect Of HHT888-45* On Type C Hepatitis Patients

	SGOT**, unit/mL		SGPT**, unit/mL	

Patient	Before	After	Before	After	Duration (days)
1	112	53	238	146	3
		30		35	64
		16		18	77
2	81	35	103	62	9
		41		61	20
		46		67	29
		32		56	37
		21		43	53
		24		50	70
		23		43	85
		28		55	102
		23		44	117
		23		29	178
3	117	96	179	123	8
		75		74	19
		66		69	26
		47		51	34
		55		48	42
		42		45	50
		48		40	70
		38		32	79
		30		26	88
4	48	32	71	65	56
		30		55	70
		21		37	87
5	83	64	67	54	8
		58		46	14
		56		40	22
		42		34	29
		38		28	36
6	166	106	291	206	2
		71		121	16
		51		81	22
		57		89	29
		36		45	45
		31		36	50
		28		37	58
		22		29	64
		28		32	71
		25		27	85
		36		28	103
		23		27	113
		23		22	163
7	30	28	41	42	9
		29		32	17

*Comprising mainly Nos. 4(3), 4(4), 5(4) and 5(5), and occasionally 4(2) and 5(8).
**SGOT = serum glutamine oxalacetate transferase; normal range = 8-40 unit/mL.
SGPT = serum glutamine pyruvate transferase; normal range = 5-35 unit/mL.

The results clearly demonstrate that the herb mixture HHT888-45 is effective in treating hepatitis C

patients. To accomplish that, the causative HCV needs to be eradicated or reduced to a tolerable level. Since HHT888-45 components have demonstrated very strong anti-HIV in vitro activity and several of the components have demonstrated efficacy in reducing HBV in carriers, the herb mixture will therefore be effective in treating patients infected with HIV and HBV.

It is therefore an aspect of this invention that the antiviral herbal medicines including the herb mixtures according to this invention and their single-herb components at various proportions and effective doses are effective in treating hepatitis C, hepatitis B, and other retroviral diseases, such as AIDS.

Since a precise chemical identification and pharmacological mechanism of the compositions of this invention have not yet been elucidated, it is possible that the antiviral activity may be due to a single herbal component, a combination of components or the biological metabolite or derivative thereof. The following Examples investigate the chemical identification of the active components set forth in this application.

EXAMPLE 8

Fractionation of Active Single-Herb Components

The three most anti-HIV active single-herb components of HHT888-5: No.5(4), No.5(5) and No.5(8) were fractionated by water extraction, C18 solid-phase-extraction (SPE) column liquid chromatography (C18-SPE-LC) and C18 column high-performance liquid chromatography (C18-HPLC). The herb mixture HHT888-4 was also fractionated concurrently for comparison. The purpose was to identify the active compound or compounds of each anti-HIV active herbal medicines or medicinal herbs.

1. Water Extraction

The single-herb herbal medicines No.5(4), No.5(5) and No.5(8) and the herb mixture HHT888-4 were extracted twice with water at ambient temperature (about 25.degree. C.) with 8 to 10 mL of water per gram of sample (e.g., 5 g powder with 40 mL water and 50 g powder with 500 mL water) each time. The water suspension was stirred for fifteen (15) minutes or vortexed for one (1) minute, stood for ten (10) minutes and vortexed again for one (1) minute. The water extract was separated from the insolubles by centrifuge at 1,500 rpm for twenty (20) minutes and filtered through a Whatman No. 4 filter paper.

2. C18-SPE-LC

Each water extract was fractionated by C18-SPE column liquid chromatography. A ten (10) mL aliquot of the extract was loaded onto a 10-g ISOLUTE C18(EC)-SPE column (from International Sorbent Technology, Ltd., Hengoed, Mid-Glamorgan, UK or Jones Chromatography, Lakewood, Colo., USA) which was preconditioned with 50 mL ethanol and 100 mL water. The SPE column (2.6 cm inside diameter by 2.7 cm length, plus 60 mL of reservoir) was packed with 10 g of end-capped (EC) C18 sorbent particles with an average particle diameter of 61 .mu.m and carbon loading of 19%.

The loaded column was eluted in sequence and by gravity with 90 mL water, 100 mL ethanol, 100 mL 1% HCl in ethanol, and 50 mL 0.1% HCl in ethanol/water at 10/90, v/v. Eluate was collected in 50-mL samples. The water eluates were either freeze-dried in a glass flask or air-dried in plastic weighing dishes. The ethanol, acidic ethanol, and acidic ethanol/water eluates were air-dried respectively in plastic weighing dishes. The air-dried acidic ethanol (1% HCl in ethanol) and acidic ethanol/water (0.1% HCl in ethanol/water at 10/90, v/v) eluates were redissolved in 1% HCl/ethanol and water, respectively; then transferred into 4-mL WISP vials and dried again under nitrogen. The dried fractions of the first 50 mL water eluate (A), the first 50 mL ethanol eluate (B), the first 50 mL 1% HCl/ethanol eluate (C), and the

50 mL 0.1% HCl/10% ethanol/90% water eluate (D) were tested for anti-HIV activities as previously described. The results are shown in Table 7.

TABLE 7

Anti-HIV Activities Of C18-SPE-LC Fractions Of HHT888-4E, No.5(4)E, No.5(5)E and No.5(8)E

Water C18-SPE-LC Extract	Fraction**	% Weight***	Test Level	Anti-HIV Activity* Toxicity**** Day 3	Day 4
HHT888-4E					
	A	70.0 .+-. 4.5%	0.7 mg/mL	50-73%	98% 61%
	B	18.5 .+-. 1.3%	0.2 mg/mL	21-95%	100% 87%
	C	6.7 .+-. 2.7%	0.1 mg/mL	98%	95% 85%
	D	2.1 .+-. 1.5%	0.1 mg/mL	100%	42% 0%
No.5(4)E					
	A	78.6 .+-. 7.6%	1.0 mg/mL	78%	99% 14%
			0.2 mg/mL	76-97%	90% 20%
	B	14.6 .+-. 3.9%	0.1 mg/mL	68%	80% 2%
			0.05 mg/mL	>68%	63% 0%
	C	4.1 .+-. 0.3%	0.1 mg/mL	91%	49% 0%
	D	3.0 .+-. 1.5%	0.1 mg/mL	96%	19% 0%
No.5(5)E					
	A	73.9 .+-. 7.9%	1.0 mg/mL	98%	100% 100%
			0.3 mg/mL	97-98%	100% 94%

		0.3 mg/mL		
		85-90%		
			99%	99%
		0.1 mg/mL		
		85%	91%	86%
	B 14.6 .+-. 4.1%			
		0.1 mg/mL		
		48%	98%	90%
		0.07 mg/mL		
		>48%	99%	41%
	C 8.6 .+-. 1.8%			
		0.1 mg/mL		
		99%	96%	96%
	D 2.5 .+-. 1.7%			
		0.1 mg/mL		
		96%	25%	0%
No.5(8)E				
	A 43.6 .+-. 2.6%			
		0.5 mg/mL		
		70%	93%	54%
		0.3 mg/mL		
		70-100%		
			96%	5%
	B 53.7 .+-. 5.0%			
		0.3 mg/mL		
		24-68%		
			100%	
				100%
		0.1 mg/mL		
		68%	88%	30%
	C 2.2 .+-. 0.9%			
		0.1 mg/mL		
		100%	49%	0%
	D 1.6 .+-. 0.5%			
		0.1 mg/mL		
		95%	14%	0%

*% suppression of HIV proliferation based on viral protein p24 levels.
**A = Water Eluate; B = Ethanol Eluate; C = 1% HCl/Ethanol Eluate; D = 0.1% HCl/10% Ethanol/90% Water Eluate.
***Determined from three to five runs.
****Toxicity in percent of control proliferation.

Table 7 sets forth the cytotoxicity and anti-HIV activity test results of C18-SPE-LC fractions A, B, C and D of HHT888-4E, No.5(4)E, No.5(5)E and No.5(8)E.

The average % weight and the standard deviation of each C18-SPE-LC fraction for the water extract (E) of each sample determined from three (3) to five (5) runs are also shown in Table 7. The sample load per column per run was 199 to 205 mg for HHT888-4E, 94 to 95 mg for No.5(4)E, 124 to 130 mg for No.5 (5)E, and 165 to 178 mg for No.5(8)E on dry weight basis.

The results show that the water eluate (A) and the 1% HCl/ethanol eluate (C) fractions of No.5(5)E and the 1% HCl/ethanol eluate fraction (C) of HHT888-4E are the most active ones (91 to 96% suppression of HIV proliferation on day 3 and 85 to 96% suppression on day 4 at 0.1 mg/mL) and noncytotoxic (85 to 99% of control). Air drying did not affect the activity of the water eluate fraction (A) of No.5(5)E. An

air-dried No.5(5)E-A exhibited an activity of 99% suppression on both day 3 and day 4 at 0.3 mg/mL and 91% suppression on day 3 and 86% suppression on day 4 at 0.1 mg/mL. As a comparison, a freeze-dried No.5(5)E-A exhibited an activity of 100% suppression on both day 3 and day 4 at 1.0 mg/mL and 100% suppression on day 3 and 94% suppression on day 4 at 0.3 mg/mL.

The ethanol eluate fractions (B) of HHT888-4E, No.5(5)E, and No.5(8)E also exhibited good anti-HIV activities: 100% suppression on day 3 and 87% suppression on day 4 at 0.2 mg/mL for HHT888-4E, 98% suppression on day 3 and 90% suppression on day 4 at 0.1 mg/mL for No.5(5)E, and 100% suppression on both day 3 and day 4 at 0.3 mg/mL for No.5(8)E. However, the observed activities may be attributed partly to the cytotoxicity: 21 to 95% of control proliferation for HHT888-4, 48% for No.5 (5)E, and 24 to 68% for No.5(8)E. This hypothesis was supported by the significant decrease of the activity when the sample concentration was lowered to a less cytotoxic level. For example, the activity decreased from 90% to 41% inhibition on day 4 for No.5(5)E-B when its concentration was decreased from 0.1 to 0.07 mg/mL. The activity decreased from 100% to 30% inhibition on day 4 for No.5(8)E-B when its concentration was decreased from 0.3 to 0.1 mg/mL.

The water eluate fractions (A) of HHT888-4E and No.5(8)E exhibited moderate anti-HIV activity: 98% suppression on day 3 and 61% suppression on day 4 at 0.7 mg/mL for HHT888-4E and 93% suppression on day 3 and 54% suppression on day 4 at 0.5 mg/mL for No.5(8)E. However, the activity may be partly attributed to cytotoxicity, which was 50 to 73% for HHT888-4E and 70% for No.5(8)E. The activity of No.5(8)E-A decreased from 54% to 5% on day 4 when the concentration decreased from 0.5 to 0.3 mg/mL where the cytotoxicity level (70-100% of control) was more acceptable.

The water eluate fraction (A) of No.5(4)E was marginally active: 90 to 99% suppression on day 3 and 14 to 20% suppression on day 4 at 0.2 to 1.0 mg/mL, respectively. The ethanol eluate fraction (B) of No.5(4)E, 1% HCl/ethanol eluate fractions (C) of Nos. 5(4)E and 5(8)E, and 0.1% HCl/10% ethanol/90% water eluate fractions (D) of all four samples were essentially not active at the levels tested: 14 to 80% suppression on day 3 and 0 to 2% suppression on day 4 at 0.05 to 0.1 mg/mL.

Additional runs of C18-SPE-LC fractionation of No.5(5)E were conducted to produce more No.5(5)E-A and C fractions for further evaluation. Water extract (E) of No.5(5) was either loaded directly (10 mL per column), or was freeze-dried or air-dried first, redissolved in water (20 to 80 mg/mL), and then loaded (5 mL per column) for the fractionation. The overall % weight distribution of each fraction from these runs of No.5(5)E was: A=74.2.+-.6.0%, B=12.2.+-.4.0%, C=8.2.+-.2.1%, and D=2.1.+-.0.9%. These values are in good agreement with those shown in Table 7.

3. C18-HPLC

The above air-dried No.5(5)E-A was further fractionated by C18-HPLC. A 500.9 mg sample was dissolved in 5.00 mL water which was then centrifuged at 1500 rpm for twenty (20) minutes to separate the solution from the insolubles. The supernate was filtered through a 0.45-.mu.m filter to remove any insoluble residues and was designated the water soluble fraction (WS). The precipitate was extracted with 5.00 mL of water three more times and nitrogen dried as the water insoluble fraction (WI). The water soluble fraction (WS) was fractionated by a gradient HPLC using a Rainin Dynamax C18 preparatory column (21.4.times.250 mm, 5 .mu.m particles) and the following conditions:

Flow rate:	9.00 mL/min
Injection Volume:	200 .mu.L
Detection:	UV 214 nm at 2.00 AUFS
Gradient:	Time Acetonitrile/Water

```
0-10 min   2/98
10-25 min  2/98 .fwdarw. 98/2 (linear)
25-30 min  98/2
30-35 min  98/2 .fwdarw. 2/98 (linear)
35-70 min  2/98
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Fractions were collected at 2.5 min intervals. A total of 28 fractions were collected at 22.5 mL for each fraction. Table 8 indicates that certain fractions were pooled before conducting the assay.

Each of the above nitrogen dried C18-HPLC fractions was tested for anti-HIV activity at a concentration equivalent to 0.33 mg/mL of the starting material, i.e., the water soluble fraction (WS) of the air-dried No.5(5)E-A. The purpose was to identify the active fraction or fractions of the air-dried No.5(5)E-A which tested very active: 99% suppression of HIV proliferation at 0.3 mg/mL (see Table 7). At the concentration equivalent to 0.33 mg/mL of the starting material, any active fraction was expected to also have a very good activity of 99% suppression. The first water extract (WS) and the precipitate (WI) of the air-dried No.5(5)E-A were also tested concurrently at 0.33 mg/mL. Table 8 sets forth the results. The % weight of each fraction is included and Fraction 3 contains the main peak.

TABLE 8

Anti-HIV Activities Of Water Soluble And Insoluble Fractions Of Air-Dried No. 5(5)E-A And The HPLC Fractions Of The Water Soluble Fraction

No. 5(5)B-A Fractions*	% Weight**	Toxicity***	Anti-HIV Activity**** Day 3	Day 4
Water Insoluble (WI)	10.3%	100%	100%	100%
Water Soluble (WS)	86.0%	100%	86%	63%
WS-HPLC-F1 & 2	6.8%	86%	0%	4%
WS-HPLC-F3	109%	73%	0%	1%
WS-HPLC-F4	2.7%	93%	8%	0%
WS-HPLC-F5	2.0%	100%	0%	0%
WS-HPLC-F6	0.7%	100%	15%	0%
WS-HPLC-F7	<0.2%	100%	46%	0%
WS-HPLC-F8	3.0%	100%	0%	0%
WS-HPLC-F9	<0.2%	100%	0%	0%
WS-HPLC-F10	<0.2%	87%	0%	1%
WS-HPLC-F11 & 12	0.7%	91%	1%	1%
WS-HPLC-F13 & 14	10%	96%	0%	0%
WS-HPLC-F15 & 16	7.0%	89%	18%	0%
WS-HPLC-F17 & 18	1.5%	77%	25%	3%
WS-HPLC-F19 to 28	1.2%	80%	0%	0%

```
*WI and WS were water insoluble and soluble fractions of the airdried No.
5(5)EA. WSHPLC-F1 to 28 were the HPLC fractions of WS.
**% Weight of the starting material (WS)
***Toxicity in % of control proliferation.
****Activity in % supppression of HIV proliferation based on viral protei
p24 level. The test levels for WI and WS of the airdried No. 5(5)EA were
both 0.33 mg/mL. The test levels for all HPLC fractions of WS were
equivalent to 0.33 mg/mL of the starting material WS.
```

Table 8 shows that fraction WS-HPLC-F3 contained the most material, however, it was essentially not active in the anti-HIV assay.

These results are very surprising. All C18-HPLC fractions tested were essentially inactive, 0 to 4% suppression on day 3 and 0-4% suppression on day 4. The water soluble fraction (WS) also showed a significantly lower activity (86% suppression on day 3 and 63% suppression on day 4 at 0.33 mg/mL) than expected. This type of activity loss during separation and purification of active components from medicinal plants has been widely experienced by others, mostly attributing to the loss of synergistic effects when the compounds are separated from their matrix.

Our results indicated that the active component was surprisingly left behind in the water insoluble fraction (WI), instead of in the water soluble fraction (WS) as originally expected. The insoluble fraction tested very active, 100% suppression on both day 3 and day 4 at 0.33 mg/mL. That means the main active component of the air-dried No.5(5)E-A is in the water insoluble fraction (WI), instead of in the soluble fraction (WS).

The active component of No.5(5)E-A was originally soluble in water since it was in the C18-SPE-LC water eluate fraction. It had to become insoluble in water during the air drying and thus remained in the water insoluble fraction. The water insoluble fraction then must have become soluble in the neutral cell culture medium for it to be tested active. This must be the case as the sample solution in the cell culture medium was centrifuged and 0.45-.mu.m filtered to remove insoluble substances before the anti-HIV assay.

EXAMPLE 9

Identification of Active Components

1. No.5(5)E-A

The pH of the cell culture medium used to dissolve the sample for anti-HIV assay was 7.3.+-.0.3. It was therefore hypothesized that the active component of No.5(5)E-A was soluble in neutral aqueous solution, like the cell culture medium, but became acidified and thus insoluble upon exposure to the atmosphere containing HCl vapor during the air drying in a hood together with other C18-SPE-LC fractions that contained HCl. That means the acid form of the active component of No.5(5)E-A would be insoluble in water and precipitate when acidified. No.5(5)E-A is the C18-SPE-LC water eluate fraction of No.5(5) water extract.

To test the hypothesis, we tested the solubility of the above active precipitate (WI) from No.5(5)E-A in various solvents. The precipitate was slightly soluble in water and acidic ethanol solutions, such as 1% HCl in ethanol and 0.1% HCl in ethanol/water (10/90, v/v), and formed very light to light yellow solutions with dark brown precipitates. It was not soluble in methanol, acetone, and 1% hydrochloric

acid. It was soluble but slowly in neutral phosphate buffer saline (PBS, pH 7.2) which became a dark brown solution overnight. It was rapidly and completely solubilized in 1% ammonium hydroxide solution (pH of 10.4) which quickly became a dark brown solution. This confirmed the above hypothesis that the active component was soluble in neutral or alkaline solutions but insoluble in acid solution.

Based on the solubility test, the active precipitate (WI) of No.5(5)E-A should be an acid or acids. The fact that it was not soluble in alcohols (methanol, ethanol, isopropanol), acetone, and other common organic solvents (acetonitrile, chloroform, and hexane) suggests that it is unlikely to be a simple organic acid, such as benzoic acid. The fact that it became progressively more soluble in aqueous solutions with an increase of pH indicated a transition from its acid form to a more soluble salt form.

To more definitively identify the active component of No.5(5)E-A, three water eluate fractions of No.5 (5)E which were dried differently were extracted with water the same way as that of the air-dried No.5 (5)E-A used for the above HPLC fractionation. One fraction was freeze-dried (FD) and two were air-dried (AD1 & AD2). AD1 and FD were prepared from the same water eluate. AD2 was the one whose activity was surprisingly found in the water insoluble fraction (WI).

Each sample was dissolved in water at 100 mg/mL and centrifuged (1,500 rpm for 20 min) to separate the soluble from the insoluble. For FD and AD1, 1.20 g of the sample was dissolved in 12.0 mL water. For AD2, 0.567 g was dissolved in 5.67 mL water. Each precipitate was extracted again with the same amount of water three more times. A 0.40 mL aliquot of each extract was nitrogen-dried and weighed. The remaining extracts were each 0.45-.mu.m filtered, acidified with 1% HCl (e.g., 4 mL acid to 10 mL extract), and centrifuged (2,000 rpm for 20 min). The acid supernate (AS) was 0.45-.mu.m filtered. The acid precipitate (AP) was washed with 10 mL 0.01% HCl (pH 2.82) two times and 10 mL water two times, nitrogen-dried and weighed.

Table 9 shows the pH of each water extract of the freeze dried (FD) and air dried (AD1, AD2) No.5(5) E-A's, the percent (%) weight distribution of each extract and the precipitate, acid precipitate formation of each extract, and the % weight of the combined acid precipitate from each No.5(5)E-A.

TABLE 9

Water Extracts, Water Precipitates And Acid Precipitates Of Freeze Dried (FD) And Air Dried (AD1, AD2) No. 5(5)E-A's

No. 5 (5)E-A	Fractions*	Solution pH	% Weight	Acid Precipitation	Acid Precipitate % Weight
FD	1st Water Extract	5.80	86.8%	Yes	8.5%**
	2nd Water Extract	6.10	2.8%	Some	
	3rd Extract	5.79	0.3%	Trace	
	4th Extract	5.85	<0.3%	No	
	Water precipitate	--	0.9%	--	
AD1	1st Water Extract	4.83	84.0%	Yes	8.3%**
	2nd Water Extract	5.14	3.8%	Some	
	3rd Water Extract				

		5.53	0.8%	No	
	4th Water Extract				
		5.44	<0.3%	No	
	Water precipitate				
		--	3.1%	--	
AD2	1st Water Extract				
		3.21	77.3 .+-.		
				No	0.3%***
			3.2%		
	2nd Water Extract				
		3.51	6.9 .+-.		
				No	
			0.8%		
	3rd Water Extract				
		3.87	2.3 .+-.		
				Some	
			1.1%		
	4th Water Extract				
		3.93	0.3 .+-.		
				Some	
			0.4%		
	Water precipitate				
		--	9.4 .+-.		
				--	
			1.3%		

*At 100 mg/mL of No. 5(5)EA in water.
**Acid precipitates from the 1st and 2nd extracts combined.
***Acid precipitate from the 3rd and 4th extracts combined.

It was clearly shown that the water extracts of AD2 were more acidic (pH's 3.2 to 3.9) than those of AD1 (pH's 4.8 to 5.5) and FD (pH's 5.8 to 6.1). The AD2 sample contained more water insoluble substance (9.4%) than AD1 (3.1%) and FD (0.9%). The more acidic the water extracts the greater amount of the insoluble substance isolated.

When the water extracts were acidified, the first and second extracts of FD and AD1 formed precipitate, while those of AD2 did not. Instead, some precipitate formed in the 3rd and 4th extracts of AD2 whose original pH was 3.9. A trace precipitate was observed in the acidified 3rd extract of FD, while no precipitate was observed in the 4th extract of FD and in the 3rd and 4th extracts of AD1.

This indicated that the active precipitate was insoluble in water at pH 3.2 to 3.5, slightly soluble at pH 3.9, and became soluble at pH 4.8 and above. Most FD was soluble in water whose solution pH was 5.8 to 6.1. Only 0.9% remained insoluble. AD1, whose solution pH was 4.8-5.5, contained a bit more insoluble material or precipitate, 3.1%. AD2, whose solution pH was 3.2 to 3.9, contained even more insoluble material or precipitate, 9.4%. When the water extracts of FD and AD1 were acidified, precipitate formed (8.3 to 8.5%). The total precipitate of FD (9.4%) or AD1 (11.4%) was comparable to that of AD2 (9.7%). The % weight of the acid supernate from the first extract of freeze dried No.5(5)E-A or FD was 78.4%, which accounted for the balance of the material.

The first water extract and the precipitate of FD, and the acid supernate and the acid precipitate of the first water extract of FD were tested for anti-HIV activity. The results are shown in Table 10, which clearly indicated that the active component of FD or freeze-dried No.5(5)E-A was originally soluble in water and precipitable by acid. The main activity of FD was in the water extract (89% suppression on

day 3 and 96% suppression on day 4 at 0.3 mg/mL) and not in the precipitate (13% suppression on both day 3 and day 4 at 0.3 mg/mL). The main activity of the water extract, in turn, was in the acid precipitate (97% suppression on day 3 and 98% suppression on day 4 at 0.3 mg/mL) and not in the acid supernate (4% suppression on day 3 and 22% suppression on day 4 at 0.3 mg/mL). The cytotoxic factor of the water extract (cytotoxicity: 75% of control at 0.3 mg/mL) apparently remained soluble in acid (cytotoxicity: 60% of control at 0.3 mg/mL) and was separable from the active acid precipitate (cytotoxicity: 90% of control at 0.3 mg/mL).

TABLE 10

Anti-HIV Activities Of Freeze Dried No. 5(5)E-A (FD) Fractions

FD Fractions	% Weight	Test Level (mg/mL)	Toxicity*	Anti-HIV Activity** Day 3	Day 4
1st Water Extract	86.8%	0.3	75%	89%	96%
Water Precipitate	0.9%	0.3	90%	13%	13%
1st Water Extract HCl Supernate	78.4%	0.3	60%	4%	22%
1st Water Extract HCl Precipitate	8.5%	0.3	90%	97%	98%

*Toxicity in % of control proliferation.
**Activity in % suppression of HTV proliferation based on viral protein p24 level.

It was therefore hypothesized that the active component of the freeze dried No.5(5)E-A was essentially the same as that of the air dried No.5(5)E-A, and both were insoluble in acid. When No.5(5)E-A was freeze-dried, the active component remained soluble in water and became insoluble when the solution was acidified. When No.5(5)E-A was air dried, part or all of the active component was acidified and became insoluble, as in the case of AD1 and AD2. The source of acid was the HCl vapor from the acidic ethanol/water fractions, since the water and ethanol eluates (A and B) were air dried along with the acidic ethanol/water eluates (C and D) in the same hood.

To verify the hypothesis, the active water precipitate of AD2 was redissolved in a neutral and a basic solution and reprecipitated with acid. Thus, two 50-mg samples of the precipitate were each dissolved in 40 mL of PBS buffer a neutral (pH 7.2) or a basic 1% NH.sub.4 OH solution (pH 10.4). The dissolution of the sample was slow in the PBS buffer and rapid in the 1% ammonium hydroxide solution. Both samples were not completely solubilized even after being stored overnight in a refrigerator. The solutions were then centrifuged at 1,500 to 2,000 rpm for 40 minutes to separate the soluble from the insoluble. Each supernate was filtered through a 0.45-.mu.m filter. Each brown precipitate was washed with 10 mL of its respective solvent and then 10 mL of water. The washes were separated by centrifuge at 2,000 rpm for 20 minutes and were discarded. Each washed precipitate and a 4.00 mL aliquot of each filtered supernate were nitrogen-dried and weighed. A 4.00 mL PBS buffer was also dried concurrently for solvent blank correction of the PBS supernate.

Two 17.0 mL aliquots of each of the supernates were pipetted into separate 50-mL centrifuge tubes. One

aliquot was acidified by titration with 1% HCl until a precipitate formed. The other was acidified by titration with 1% acetic acid first and then with 1% HCl until it formed a precipitate. Titration with 1% acetic acid alone was insufficient to bring down the solution pH low enough to form precipitate. The solution pH titrated with 1% acetic acid leveled off at a pH of about 3.4 to 3.8 with no visible precipitate. Addition of 1% HCl was needed to bring the solution pH lower to around 1.5 to 1.8 to form precipitate. Visible precipitate began to form at solution pH around 2.2 to 2.5. When the supernates were titrated with 1% HCl, the solution pH's were lowered to 1.4 and 1.5 and precipitates formed. Precipitate began to form at pH around 2.3 for PBS supernate and around 3.3 for NH.sub.4 OH supernate titrated with HCl.

The acid supernate was separated from the acid precipitate by centrifuge at 2,000 rpm for 20 minutes. Each acid supernate (AS) was filtered through a 0.45-.mu.m filter and nitrogen dried. Each acid precipitate (AP) was washed with 5 mL of 1% HCl once and nitrogen dried. The PBS supernate and precipitate, the acid (HCl) supernate and precipitate of the PBS supernate, and the acid (HCl) precipitate of the NH.sub.4 OH supernate were tested for anti-HIV activities. The results are shown in Table 11.

TABLE 11

Anti-HIV Activities Of Fractions Of The Active Air Dried No. 5(5)E-A Water Insoluble Fraction (AD2-WI)

AD2-WI Fractions	% Weight	Test Level (mg/mL)	Toxicity*	Anti-Hiv Activity** Day 3	Day 4
PBS Supernate	72.3%	0.3	100%	94%	98%
PBS Precipitate	28.9%	0.3	74%	26%	34%
PBS Supernate HCl Supernate	47.9%	0.3	50%	60%	65%
PBS Supernate HCl Precipitate	52.8%	0.3	100%	83%	92%
NH.sub.4 OH Supernate HCl Precipitate	50.2%	0.3	92%	93%	97%

*Toxicity in % of control proliferation.

**Activity in % suppression of HIV proliferation based on viral protein p24 level.

The results clearly evidence that the active component of AD2-WI is soluble in PBS at pH 7.2 and 1% NH.sub.4 OH solution at pH 10.4 and is precipitable by acid, such as HCl. The PBS supernate of AD2-WI is very active (94% suppression on day 3 and 98% suppression on day 4 at 0.3 mg/mL) while the PBS precipitate is marginally active (26% suppression on day 3 and 34% suppression on day 4 at 0.3 mg/mL). This is consistent with the observed activity of the AD2-WI whose active component had to be solubilized in the neutral cell culture medium for the anti-HIV assay.

The active component of AD2-WI was reprecipitated with HCl. The HCl precipitate of the PBS supernate of AD2-WI was fairly active (83% suppression on day 3 and 92% suppression on day 4 at 0.3 mg/mL) while the HCl supernate was moderately active (60% suppression on day 3 and 65%

suppression on day 4 at 0.3 mg/mL). The moderate activity of the HCl supernate may be partially due to the cytotoxicity (50% of control proliferation). The acid precipitability of the active component of AD2-WI was further confirmed by the HCl precipitate of the NH.sub.4 OH supernate of AD2-WI which was very active (93% suppression on day 3 and 97% suppression on day 4 at 0.3 mg/mL).

From this information, it can be concluded that the active component of No.5(5)E-A is soluble in neutral or basic solutions and precipitable by acids such as HCl. Acetic acid, which is only able to bring the solution pH down to around 3.4 to 3.8, is not strong enough to cause precipitation of the active component. That means the active component in its acid form is stronger than acetic acid and weaker than hydrochloric acid. When neutralized with a base, such as NH.sub.4 OH, the active insoluble acid becomes a water soluble salt, which is also active against HIV.

More of the active component was prepared by acid precipitation from the water eluate No.5(5)E-A "as is" or the water extracts of freeze dried No.5(5)E-A by titration with 1% HCl. The acid precipitate was washed with water and freeze dried. The freeze dried acid precipitate was further purified by dissolving in 0.1 N ammonium bicarbonate (NH.sub.4 HCO.sub.3) and reprecipitating it with 1.5 times volume of 1% HCl in water. For example, a 520.8 mg sample was completely solubilized in 20 mL of 0.1 N NH.sub.4 HCO.sub.3. The solution was centrifuged at 2,000 rpm for 22 min and the supernate was filtered through a 0.45-.mu.m filter. An aliquot of the solution was diluted with 0.1 N NH.sub.4 HCO.sub.3 to 20.0 mL at 18.7 mg/mL, which was then acidified with 30.0 mL 1% HCl in water and formed a dark brown fluffy suspension and precipitate. The acidified solution was centrifuged at 2,000 rpm for 22 minutes. The acid supernate was decanted and discarded. The acid precipitate was washed with 30 to 45 mL of 1% HCl in water six times. The acid washes were each separated from the precipitate by centrifuge at 2,000 rpm for 22 minutes and discarded. The once purified acid precipitate (AP1X) was freezed dried and tested for anti-HIV activity. The result showed that the AP1X of No.5(5) E-A remained active: 75% suppression on day 3 and 87% suppression on day 4 at 0.31 mg/mL. That is, the anti-HIV activity survived the purification process.

2. No.5(5)E-C

Since the active component of No.5(5)E-A was identified to be the one soluble in neutral and basic solutions and precipitable by HCl, it was logical to see whether the active component of No.5(5)E-C possessed similar characteristics.

The air-dried No.5(5)E-C contained two distinct colored solids, one brown and one dark brown to near black. A solubility test was conducted by mixing 1.1-1.2 mg of the sample in one (1) mL of each solvent tested. The air-dried No.5(5)E-C was insoluble in ethanol, isopropanol, acetone, acetonitrile, chloroform, and hexane. It was slightly soluble in methanol and partially soluble in water, 1% HCl in water, 1% HCl in ethanol, and 0.1% HCl in 10% ethanol/90% water. It was mostly solubilized in PBS, 0.1 N NH.sub.4 HCO.sub.3, 1% NH.sub.4 OH, and 1% NaOH in water with a small amount of off-white suspension or precipitate. The solubility appeared to progressively increase with the solution pH from acidic to neutral to slightly basic, and then decrease in a strong base like 1% NaOH in water.

The one-mL solutions of air-dried No.5(5)E-C in water, PBS, 0.1 N NH.sub.4 HCO.sub.3, 1% NH.sub.4 OH, and 1% NaOH were each 0.45-.mu.m filtered and acidified with 1.5 mL 1% HCl in water. All acidified solutions became yellowish brown to brown solutions and formed brown fluffy precipitates, except the acidified water solution in which no precipitate was observed.

In addition, the one-mL solutions of the air dried No.5(5)E-C in 1% HCl/water and 1% HCl/ethanol were each 0.45-.mu.m filtered and mixed with 1.5 mL 1% NaOH to make the solution alkaline. The purpose was to see if these solutions contained insoluble bases. The alkalinated 1% HCl/water solution

became a yellow-clear solution and the alkalinated 1% HCl/ethanol solution became yellowish-brown to brown solution. Both formed some golden brown fluffy precipitate after overnight storage in a refrigerator. The brown one-mL solution of No.5(5)E-C in 0.1% HCl/10% ethanol/90% water was also alkalinated the same way but with 1.5 mL of 1% NH.sub.4 OH. The brown solution became a light yellow clear solution upon alkalination precipitate did not form after overnight refrigerated storage. The results indicate that acidic and basic substances may be separated from No.5(5)E-C by precipitation with a strong acid and a strong base, respectively.

An 100.4 mg air dried sample of No.5(5)E-C was dissolved in 20.0 mL 0.1 N NH.sub.4 HCO.sub.3. The solution was centrifuged at 2,000 rpm for 20 min and the supernate was transferred into a separate 50-mL centrifuge tube. The precipitate was extracted with 15.0 mL 0.1 N NH.sub.4 HCO.sub.3 two more times. The supernates were pooled (total 50 mL). The precipitate was washed one more time with 15 mL 0.1 N NH.sub.4 HCO.sub.3. The wash was separated by centrifuge at 2,000 rpm for 30 min and discarded. The precipitate was transferred into a WISP glass vial with 1 to 2 mL of water, nitrogen dried, and weighed 0.5 mg after drying, or 0.5% of the air dried No.5(5)E-C starting material.

The supernate was filtered through a 0.45-.mu.m filter. Two 20.0 mL aliquots of the filtered supernate were pipetted into two 50-mL centrifuge tubes. The first 20-mL aliquot was acidified with 30 mL 1% HCl in water and formed a fluffy precipitate. The second 20-mL aliquot was made alkaline with 30 mL 1% NaOH. The alkalinated solution remained clear and with no visible suspension or precipitate after overnight refrigerated storage. The alkalinated solution also remained clear after being centrifuged at 2,000 rpm for 30 minutes or concentrated from 50 mL to 14 mL and then centrifuged. Addition of an additional 20 mL 1 N NaOH, to assure that the solution was alkaline (pH 13.23 at 22.5.degree. C.), did not produce any visible precipitate.

The acidified solution was centrifuged at 2,000 rpm for 30 minutes to separate the fluffy acid precipitate. The acid precipitate was washed with 20 mL 1% HCl in water three times. Each wash was separated by centrifuge at 2,000 rpm for 30 minutes and discarded. The washed acid precipitate was freeze dried, tested for anti-HIV activity, and found active: 76% suppression on both day 3 and day 4 at 0.3 mg/mL.

The air dried No.5(5)E-C was fractionated again by directly dissolving it in 1% HCl in water to prepare the acid precipitable active component. The acid supernate was made alkaline to prepare the base precipitable component. A 203.8 mg air dried No.5(5)E-C sample was dissolved in 20 mL 1% HCl in water by vortexing the suspension for one (1) minute three times. The acid soluble portion was separated from the acid insoluble by centrifuge at 2,000 rpm for 30 min. The acid supernate (reddish brown solution) was filtered through a 0.22-.mu.m filter. A 2.00 mL aliquot of the acid supernate was nitrogen dried and weighed 12.2 mg, or 59.9% of the air dried No.5(5)E-C starting material. The remaining 18 mL of the acid supernate was alkalinated with 7.5 mL of 1 N NaOH. Precipitate formed after cooling in a refrigerator for about 5 minutes. The base precipitate was separated from the base supernate by centrifuge at 2,000 rpm for 60 minutes. The base supernate was filtered through a 0.22-.mu.m filter and neutralized with 5.0 mL of 1% HCl in water to pH 3.7 (clear brown solution). A 3.00 mL aliquot of the neutralized base supernate was nitrogen dried and weighed 54.5 mg.

The acid insoluble fraction of air dried No.5(5)E-C was washed with 20 mL 1% HCl in water twice. The base precipitate was washed with 20 mL 1% NaOH once. The washed acid insoluble fraction and the base precipitate were freeze dried and weighed 66.6 mg and 18.6 mg, respectively, or 32.7% and 10.1% of the air dried No.5(5)E-C.

The dried acid insoluble fraction, acid soluble fraction, base precipitate, and neutralized base supernate were tested for anti-HIV activities along with the starting material, air dried No.5(5)E-C. The results are shown in Table 12, which also shows the % weight of each fraction of No.5(5)E-C.

TABLE 12

Anti-HIV Activities Of Air Dried No. 5(5)E-C Fractions

No. 5(5)E-C Fractions	% Wt	Test Level (mg/mL)	Toxicity**	Anti-HIV Activity* Day 3	Day 4
No. 5(5)E-C	100%	0.31	89%	78%	76%
Acid Insoluble	32.7%	0.30	91%	84%	85%
Acid Soluble	59.9%	0.31	92%	12%	19%
Base Precipitate	10.1%	0.31	90%	16%	20%
Base Supernate***	57.9%	0.30	99%	12%	10%

*Activity in % suppression of HIV proliferation based on viral protein p2 level. The data were repeated results using sample solutions stored froze for three months.
**Toxicity in % of control proliferation.
***Neutralized with 1% HCl in water to pH 3.7 and nitrogen dried. The % weight shown excluded NaCl. The test sample contained 19.5% of No. 5(5)EC fraction.

The results clearly demonstrated that the acid insoluble fraction contained the main active component of the air-dried No.5(5)E-C. The acid insoluble fraction was fairly active: 84% suppression on day 3 and 85% suppression on day 4 at 0.30 mg/mL. The acid soluble fraction was only marginally active: 12% suppression on day 3 and 19% suppression on day 4 at 0.31 mg/mL. Both the base precipitate and base supernate of the acid soluble fraction of No.5(5)E-C were also marginally active: 12 to 16% on day 3 and 10 to 20% on day 4 at 0.30 to 0.31 mg/mL.

It is therefore concluded that the active component of No.5(5)E-C, like that of No.5(5)E-A, is also soluble in neutral to slightly basic solutions but insoluble in strong acid solutions.

3. No.5(5)E

Since the active components of both the main active C18-SPE-LC fractions A and C of No.5(5)E (Table 7) have similar solubility properties (soluble in neutral to basic solutions and precipitable in a strong acid), the main active components of No.5(5)E can thus be prepared by direct acid precipitation from the water extract of No.5(5). The acid precipitate can be further purified by redissolution in 0.1 N NH.sub.4 HCO.sub.3 and reprecipitation with hydrochloric acid one or more times. The NH.sub.4 HCO.sub.3 solution of the acid precipitate was filtered through a 0.22-.mu.m or 0.45-.mu.m filter once during one of the purification cycles to remove residual insoluble particles.

A six (6) times purified acid precipitate of No.5(5)E, or No.5(5)E-AP6X, was tested for anti-HIV activity and found to remain very active, 99% suppression on day 3 and 97% suppression on day 4 at 0.25 mg/mL, as shown in Table 13. Where, the anti-HIV activities of No.5(5) and Raw No.5(5)E are shown for comparison. The percent (%) yield of No.5(5)E-AP6X from two determinations is also listed.

TABLE 13

Anti-HIV Activities Of Water Extractable (E), Acid Precipitable (AP), And Acid Soluble (AS) Fractions Of No.4(2), No.4(3), No.4(4), No.4(5), No.5(1), No.5(4), No.5(5), No.5(8), No.5(11), G, and H

Sample	Lot	% Yield**	Test Level	Anti-HIV Activity* Toxicity*** Day 3	Day 4
No.4(2)	1	100%	2.5 mg/mL	74-84%	92% 94%
No.4(2)E	1	23.7%	0.5 mg/mL	68%	62% 0%
No.4(2)E-AP	1	0.39%	0.1 mg/mL	100%	83% 83%
No.4(2)E-AS	1	23.3%	0.25 mg/mL	69-90%	0% 0%
No.4(3)	1	100%	2.5 mg/mL	75-78%	100% 100%
No.4(3)E	1	55.3%	0.5 mg/mL	92%	97% 89%
No.4(4)	1	100%	2.5 mg/mL	74-100%	100% 100%
No.4(4)E	1	22.0%	0.5 mg/mL	67%	100% 100%
No.4(4)E-AP	1	2.1%	0.1 mg/mL	69%	85% 95%
No.4(4)E-AS	1	19.9%	0.25 mg/mL	75-100%	91% 82%
No.4(5)	1	100%	2.5 mg/mL	41-79%	98% 92%
No.4(5)E	1	21.9%	0.5 mg/mL	97%	57% 5%
No.4(5)E-AP	1	0.19%	0.3 mg/mL	86-94%	86% 80%
No.4(5)E-AS	1	21.7%	0.25 mg/mL		

```
                              91-96%
                                    4%  1%
No.5(1) 1  100%   2.5 mg/mL
                              98%   73% 50%
No.5(1)E
        1  17.4 .+-. 0.3%
                   0.5 mg/mL
                              100%  37% 0%
No.5(1)E-AP
        1  0.30%  0.3 mg/mL
                              85-94%
                                    62% 74%
No.5(4) 1  100%   2.5 mg/mL
                              64%   100%
                                        100%
No.5(4)E
        1  12.8 .+-. 1.6%
                   0.5 mg/mL
                              85%   52% 0%
No.5(5) 1  100%   2.5 mg/mL
                              80-84%
                                    93% 93%
Raw No.5(5)E
        1  18.7 .+-. 2.8%
                   1.0 mg/mL
                              99%   91% 97%
No.5(5)E-AP6X
        2  1.6 .+-. 0.1%
                   0.25 mg/mL
                              63-98%
                                    99% 97%
No.5(8) 1  100%   2.5 mg/mL
                              32-59%
                                    100%
                                        100%
No.5(8)E
        1  22.3 .+-. 3.1%
                   0.5 mg/mL
                              69%   2%  24%
No.5(8)E-AP
        1  0.26%  0.1 mg/mL
                              100%  45% 61%
No.5(11)
        1  100%   2.5 mg/mL
                              100%  92% 74%
No.5(11)E
        1  53.8%  2.0 mg/mL
                              74-94%
                                    87% 73%
No.5(11)-AP
        1  4.36%  0.3 mg/mL
                              73-100%
                                    91% 87%
No.5(11)-AS
        1  49.4%  0.5 mg/mL
                              100%  84% 65%
GE-AP   1  1.0%   0.30 mg/mL
                              33%   100%
                                        100%
GE-AP6X 1  0.50%  0.25 mg/mL
```

				66-93%
				100%
				99%
HE-AP	1	--	0.30 mg/mL	
				83% 85% 88%
HE-AS	1	--	0.25 mg/mL	
				87-91%
				24% 0%
HE-AP1X	2	0.30 .+-. 0.06%		
			0.25 mg/mL	
				93-100%
				95% 90%

*Activity in % Suppresion of HIV proliferation based on viral protein p24 level.
**From single determinations, except those with .+-. standard deviations were from 2 to 3 determinations.
***Toxicity in % of control proliferation.

4. GE

The source plant G of No.5(5) was tested to see whether the same acid precipitable anti-HIV active component can be isolated directly from the plant. Dried plant G was purchased from a local herbal store in Taiwan. A 100.8 g sample was extracted "as is" with water twice by boiling the whole dried plant in 2,900 mL water for 75 to 76 minutes each time. The first (.about.500 mL after evaporation) and the second (.about.120 mL after evaporation) extracts were separated respectively from the residue by decantation and filtered through a Whatman No. 4 filter paper. The first extract was acidified with 400 mL 1% HCl in water and the second extract was acidified with 145 mL 1% HCl in water. Precipitate formed in both acidified extracts (pH 1.5 for the first and 1.4 for the second). The acid precipitate (dark near black solid) was separated from the acid supernate (dark reddish-brown solution) by centrifuge at 2,000 rpm for 30 minutes.

Part of the acid precipitate of the first extract was washed with about 30 mL 1% HCl in water; then the inside wall of the 50-mL centrifuge tube was rinsed with about 15 mL water. The acid wash and the water rinse were separated from the acid precipitate by centrifuge at 2,000 rpm for 30 minutes and discarded. The acid precipitate was nitrogen dried (GE-AP), tested for anti-HIV activity and found very active: 100% suppression on both day 3 and day 4 at 0.30 mg/mL (Table 13). However, the GE-AP is cytotoxic at this level, 33% of control, and needs to be further purified to reduce the cytotoxicity.

Additional extractions with boiling water were conducted with dried plant chips which were cut to approximately .ltoreq.1 cm long. The percent (%) of extractables from the dried plant chips determined from two lots of the plant were from 21 to 27%. The water extracts from the chipped samples were acidified with HCl to produce acid precipitates which were then purified up to six cycles of dissolution and precipitation as described above for No.5(5)E-AP6X. The six (6) times purified GE-AP6X was tested for anti-HIV activity and found as active as No.5(5)E-AP6X, as shown in Table 13. GE-AP6X exhibited 100% suppression of HIV proliferation on day 3 and 99% suppression on day 4 at 0.25 mg/mL, while No.5(5)E-AP6X exhibited 99% suppression on day 3 and 97% suppression on day 4 at the same level. Cytotoxicity test also showed close similarity between the two active components, 66-93% of control proliferation for GE-AP6X and 63-98% for No.5(5)E-AP6X at the same 0.25 mg/mL.

5. HE

Plant H (Dichondra micrantha) was originally thought to be the source plant of the single-herb herbal medicine No.5(5), since the plant has a Chinese trivial name the same as that of the herbal medicine No.5(5). See H. C. Chang, Medicinal Herbs II, Holiday Publishing Co., Taipei, Taiwan, R.O.C., 27 (1991). Plant H was thus subjected to the same water extraction and acid precipitation as for plant G described above.

Dried whole plants of Dichondra micrantha (H) were extracted with boiling water as that described above for the extraction of plant G. The water extract was filtered and acidified with HCl and a precipitate formed. The acid precipitate (HE-AP) and the acid supernate (HE-AS) were tested for anti-HIV activities. The results clearly demonstrate that the acid precipitate HE-AP is the active component of the plant H water extract, the same situation as that for plant G and No.5(5), as shown in Table 13.

The acid precipitate HE-AP exhibited good anti-HIV activity: 85% suppression on day 3 and 88% suppression on day 4 at 0.30 mg/mL. The acid supernate HE-AS was not active: 24% suppression on day 3 and 0% suppression on day 4 at 0.25 mg/mL. A one (1) time purified acid precipitate HE-AP1X from a second lot was also tested as active: 95% suppression on day 3 and 90% suppression on day 4 at 0.25 mg/mL (Table 13). The samples were not toxic at the test levels, 83% of control proliferation for HE-AP, 93 to 100% of control for HE-AP1X, and 87 to 91% of control for HE-AS.

6. No.4(2), No.4(3), No.4(4), No.4(5), No.5(1), No.5(4) and No.5(8)

Since the active components of No.5(5) and plants G and H are all extractable by water and precipitable by acid, it is logical to see whether the active components of the other anti-HIV active single-herb herbal medicines have similar properties. The active herbal medicines were therefore checked to see whether they contained acid precipitable components and, if they did, whether these components were active.

A 5.0 g sample of each single-herb herbal medicine No.4(2), No.4(3), No.4(4), No.4(5), No.5(1), No.5 (4) and No.5(8) was extracted with about 40 mL water twice in a 50-mL plastic centrifuge tube. Each extract was separated from the insoluble material by centrifuge at 2,000 rpm for 40 to 120 minutes. The first and second extracts of each sample were combined and then filtered through a 0.22-.mu.m filter. A 2.00 mL aliquot of each extract was nitrogen dried and weighed. The remaining extract of each sample was acidified with 10 mL 1% HCl in water. Precipitates formed in all acidified extracts, except those of No.4(3) and No.5(4). No precipitate formed in the acidified extract of No.4(3), even after prolonged (9 hrs) storage in a refrigerator and addition of 10 mL more 1% HCl in water. The acidified extract of No.5 (4) showed only cloudiness and formed a trace precipitate after centrifuge at 2,000 rpm for 20 minutes.

Each acid precipitate was separated from its supernate by centrifuge at 2,000 rpm for 20 minutes. Each acid precipitate was washed with 5 mL 1% HCl in water. The acid wash was separated by centrifuge at 2,000 rpm for 20 minutes and discarded. Each acid precipitate was nitrogen dried and weighed.

Each acid supernate was combined with 10 mL more 1% HCl in water. After being stored for four (4) days at ambient temperature, precipitates formed again in various degrees in all further acidified supernates, except that of No.4(3). Each acid supernate was separated from the precipitate by centrifuge at 2,000 rpm for 80 minutes, filtered through a 0.22-.mu.m filter, and air dried. Each dried acid supernate was redissolved in 0.1 N NH.sub.4 HCO.sub.3, transferred into a WISP glass vial, and freeze dried.

The dried water extracts (E), acid precipitates (AP), and acid supernates (AS) of No.4(2), No.4(4) and No.4(5) were tested for anti-HIV activities. The water extracts (E) and acid precipitates (AP) of No.5(1) and No.5(8) were also tested. As No.4(3) did not have acid precipitate and No.5(4) had only a minute amount of acid precipitate (0.3 mg), only their water extracts (E) were tested for anti-HIV activities. The

results are shown in Table 13.

The results show that the water extracts (E) of No.4(3) and No.4(4) remain very active: 97% suppression on day 3 and 89% suppression on day 4 for No.4(3)E and 100% suppression on both day 3 and day 4 for No.4(4)E at 0.5 mg/mL. The activities of the water extracts (E) of No.4(2), No.4(5), No.5(1), No.5(4) and No.5(8), however, were surprisingly low: 2 to 62% suppression on day 3 and 0 to 24% suppression on day 4 at the same test level 0.5 mg/mL. As a comparison, the original herbal medicine powders have moderate to very good activities: 73 to 100% suppression on day 3 and 50 to 100% suppression on day 4 at 2.5 mg/mL.

Even more surprising, all acid precipitates (AP) exhibited moderate to good anti-HIV activities: 83% suppression on both day 3 and day 4 for No.4(2)E-AP, 85% suppression on day 3 and 95% suppression on day 4 for No.4(4)E-AP, 86% suppression on day 3 and 80% suppression on day 4 for No.4(5)E-AP, 62% suppression on day 3 and 74% suppression on day 4 for No.5(1)E-AP, and 45% suppression on day 3 and 61% suppression on day 4 for No.5(8)E-AP at 0.1 to 0.3 mg/mL. The acid supernate (AS) of No.4 (4)E was fairly active, 91% suppression on day 3 and 82% suppression on day 4 at 0.25 mg/mL. The acid supernates (AS) of No.4(2)E and No.4(5)E were practically inactive: 0 to 4% suppression on day 3 and 0 to 1% on day 4 at 0.25 mg/mL.

Since the water extracts (E) of No.4(2), No.4(5), No.5(1), No.5(4) and No.5(8) are much less active than their original powders and the acid precipitates (AP) of No.4(2), No.4(5), No.5(1) and No.5(8) are fairly active, it is therefore hypothesized that the majority of the active components of these powders may not have been effectively extracted into water (pH 4.0 to 5. 1). Adjustment of the solution pH to neutral or slightly alkaline is expected to help improve the extraction.

It is concluded that the active components of No.4(2) and No.4(5) are precipitable by acid. The active component of No.4(3) is soluble in both water and acid. No.4(4) contains two active components, one acid soluble and one acid precipitable. No.5(1) and No.5(8) contain acid precipitable active components.

7. No.5(11)

The single-herb components No.5(10) and No.5(11) were included in the herb mixture HHT888-5 for treating HBV carriers (See Example 3). Both No. 5(10) and No. 5(11) were not included in the earlier anti-EMuLV and anti-HIV screening tests to prevent their potential interference with the antiviral assays of HHT888-5.

The above discoveries show that all the acid precipitable components or acid precipitates isolated from the single-herb herbal medicines and medicinal plants No.4(2), No.4(4), No.4(5), No.5(l), No.5(5), No.5 (8) and H are anti-HIV active. It is therefore projected that acid precipitable components or acid precipitates, if any, isolated from other herbal medicines or plants will also be anti-HIV active.

To test the hypothesis, the single-herb herbal medicines No.5(10) and No.5(11) are extracted with water and their water extracts are acidified with HCl to see if acid precipitates will form. A 90.4 g sample of No.5(10) was extracted with twenty (20) times water (1804 to 1808 mL) at ambient temperature twice, followed by extraction with 900 mL 0.1 N NH.sub.4 HCO.sub.3 once. A 90.8 g sample of No.5(11) was extracted with ten (10) times water (908 mL) at ambient temperature twice, followed by extraction with 870 mL 0.1 N NH.sub.4 HCO.sub.3 once. The extractions were conducted in a 2000-mL glass Erlenmeyer flask and stirred magnetically for one (1) hour to overnight. The extract was separated from the insoluble by centrifuge at 8,000 rpm for 40 minutes.

When 1.0 mL of extract was acidified with 1.5 mL of 1% HCl in water, the two water extracts and the NH.sub.4 HCO.sub.3 extract of No.5(10) showed no visible precipitates even after overnight refrigerated storage. The first water extract of No.5(11) became brown and formed precipitate almost immediately. The second water extract of No.5(11) became light yellowish and slightly opaque, and formed a small amount of precipitate after overnight storage at ambient temperature. The NH.sub.4 HCO.sub.3 extract of No.5(11) became nearly colorless and with white colloidal precipitate. It was predicted that No.5(11) and its acid precipitate were likely anti-HIV active.

A 10.0 mL aliquot of each extract was freeze dried and weighed. The total extractable of No.5(10) was 66.3%. That of No.5(11) was 53.8%. Most of the extractable of No.5(10) was extracted by the two water extractions which constituted 97.9% of the total extractable. Most of the extractable of No.5(11) was extracted in the first water extraction (93.5%). The second water extraction was necessary for No.5(10), which constituted 31.2% of the total extractable. For No.5(11), the second water extraction and the NH.sub.4 HCO.sub.3 extraction constituted only 5.4% and 1.1%, respectively.

The remaining 868 mL of the first water extract, 898 mL of the second water extract, and 828 mL of the NH.sub.4 HCO.sub.3 extract of No.5(11) were acidified with 14.6, 14.6, and 13.4 mL of concentrated HCl (37%), respectively. The acidified first water extract formed precipitate almost immediately. The acidified 2nd water extract and NH.sub.4 HCO.sub.3 extract became cloudy and formed precipitate after overnight refrigerated storage. The acid precipitate (AP) of each acidified extract was separated from its acid supernate (AS) by centrifuge at 2,000 rpm for 40 minutes. The acid supernates of the first and the second water extracts were pooled in a 2000-mL glass Erlenmeyer flask. A 200 mL sample of each of the acid supernates from the acidified water extracts and NH.sub.4 HCO.sub.3 extract was centrifuged at 8,000 rpm for 40 minutes and filtered through a 0.22-.mu.m filter. A 20.0 mL of each micro-filtered acid supernate was nitrogen dried, redissolved in water, and freeze dried.

The acid precipitates of No.5(11)E from the acidified first and second water extracts and the acidified NH.sub.4 HCO.sub.3 extract were rinsed with about 20 to 40 mL water twice. The water rinses were separated by centrifuge at 2,000 rpm for 40 minutes and discarded. The acid precipitates were freeze dried and weighed. The percent (%) yield of the acid precipitate was 4.36% of No.5(11). Most (96.3%) of the acid precipitate was isolated from the original powder by the first water extraction.

No.5(10), No.5(11), the water extract of No.5(10) or No.5(10)E, the first water extract of No.5(11) or No.5(11)E, the acid precipitate from the acidified first water extract of No.5(11) or No.5(11)E-AP, and the acid supernate from the acidified pooled first and second water extracts of No.5(11) or No.5(11)E-AS were tested for anti-HIV activities. The results (Table 13) show that No.5(10) and its water extract No.5(10)E are essentially not active: 65% suppression on day 3 and 0% suppression on day 4 for No.5 (10) at 2.5 mg/mL and 0% suppression on both day 3 and day 4 for No.5(10)E at 2.0 mg/mL. No.5(11), its water extract No.5(11)E, and the acid supernate No.5(11)E-AS are moderately active: 92% suppression on day 3 and 74% suppression on day 4 for No.5(11) at 2.5 mg/mL; 87% suppression on day 3 and 73% suppression on day 4 for No.5(11)E at 2.0 mg/mL; and 84% suppression on day 3 and 65% suppression on day 4 for No.5(11)E-AS at 0.5 mg/mL. The acid precipitate No.5(11)E-AP is again fairly active: 91% suppression on day 3 and 87% suppression on day 4 at 0.3 mg/mL.

The results show that No.5(11) contains two active components, one is soluble in acid and one is precipitable by acid. Both active components are extractable from No.5(11) by water. This supports an additional aspect of the invention, that being all acid precipitable components or acid precipitates from selected plants as recited in this application, and possibly any plant, are effective pharmaceutical agents.

EXAMPLE 10

Isolation of Active Components

Active components have been isolated from either commercial extract powders or the plants by extraction with water. Preferably, the amount of water to dried plant material can range from 5 to 10 times (v/w) and at least two extractions are conducted. The pH of the extraction solution is preferably adjusted with an alkaline solution, such as a NaOH, to neutral or slightly alkaline (7 to 8) to facilitate the extraction. The extraction may be conducted at either ambient temperature (commercial powders) or boiling (chipped or pulverized plants) for one hour or longer.

The soluble extract can be separated from the insoluble plant material by filtration (i.e., nylon screen and filter paper) or by centrifuge (2,000 to 8,000 rpm for 40 minutes or longer). The extract is then acidified with any strong acid, such as HCl (approximately 0.6% final concentration or solution pH .ltoreq.2) to produce the active acid precipitate. The acid precipitate can be separated by centrifuge such as at 8,000 rpm for 40 minutes or longer. The precipitate can be purified by repetitive cycles of dissolution in neutral or alkaline solution such as 0.1 N NH.sub.4 HCO.sub.3 and subsequent precipitation in acid. The insolubles can be removed by centrifuge (such as at 8,000 rpm for 40 minutes or longer) and/or microfiltration (such as through 0.2 to 0.45-.mu.m filter).

The purified acid precipitate can be freeze dried, nitrogen dried, or air dried. It can also be converted to ammonium salt by dissolving the acid in an ammonium solution such as ammonium bicarbonate or ammonium hydroxide solution which is then freeze dried or spray dried. The purified acid precipitate can also be converted to other salts, such as sodium salt, by dissolving the acid in a suitable solution, like NaHCO.sub.3. The acid precipitate can also be separated from the matrix by C18 column chromatography.

Chemically related water extractable and acid precipitable anti-HIV active components were isolated from seven (7) of the anti-HIV active single-herb herbal medicines: No.4(2), No.4(3), No.4(5), No.5(1), No.5(5), No.5(8), No.5(11) and two (2) medicinal plants Aeginetia indica (G) and Dichondra micrantha (H) identified in Examples 5 and 6 by the water extraction and acid precipitation procedure described above.

As a specific example, No.4(2), No.4(3), No.4(4), No.4(5), No.5(1), No.5(4), No.5(5), No.5(8) and No.5 (11) which were prepared according to Example 2 were extracted twice with water at ambient temperature (about 25.degree. C.) at 8 to 10 mL of water per gram of sample (e.g., 5 g powder with 40 mL water or 50 g powder with 500 mL water or 100 g powder with 1000 mL water) each time. The water suspension was mixed to extract by either vortexing for one (1) minute, standing for ten (10) minutes and vortexing for one (1) minute; or stirring magnetically for fifteen (15) minutes or longer depending on the sample size and suspension volume. For example, the suspension containing 5 g powder in 40 mL water was vortexed for one (1) minute, stood for ten (10) minutes and vortexed again for one (1) minute during the extraction. The suspension containing 50 to 100 g powder in 500 to 1000 mL water was stirred magnetically for fifteen (15) minutes or longer during the extraction. The water extract was separated from the insoluble materials by centrifuge at 1,500 to 8,000 rpm for twenty (20) to forty (40) minutes and filtration through a filter such as Whatman No.4 filter paper.

The medicinal plants Aeginetia indica (G) and Dichondra micrantha (H), or the source plants of the herbal medicines No.5(5) and H, were washed with cold water, dried, comminuted, and extracted with boiling water as described above in Example 2. The water extracts were cooled to ambient temperature and separated from the insoluble plant materials by decantation and filtration through a nylon screen (1.3 by 1.5 mm openings), centrifuge at 1,500 to 8,000 rpm for twenty (20) to forty (40) minutes, and filtration again through a Whatman No.4 filter paper.

The water extracts were then acidified to form precipitates through the addition of hydrochloric acid to a pH of <2. Each acid precipitate was separated from the acid solution by centrifuge in plastic centrifuge tubes or bottles. Each acid precipitate was washed at least three times with water or 0.1 or 1% hydrochloric acid. Samples of the water extract, acid precipitate (acid insoluble component) and acid supernate (acid soluble component) of each of the samples were nitrogen dried, air dried or freeze dried. These samples were then subjected to further testing and characterization.

The purified acid precipitates No.5(5)E-AP1X, No.5(5)E-AP6X, GE-AP1X, GE-AP2X, GE-AP6X, HE-AP1X, HE-AP6X, No.4(2)E-AP1X, No.5(8)E-AP1X, No.5(11)E-AP1X, and their ammonium salts No.5(5)E-AP1X-NH.sub.4, GE-AP2X-NH.sub.4, HE-AP1X-NH.sub.4, No. 5(8)E-AP1X-NH.sub.4, No.5(11)E-AP1X-NH.sub.4 and No.4(2)E-AP1X-NH.sub.4 were thus prepared as described above. The nomenclature used herein and in the claims can be illustrated by the following: No.5(5)E-AP1X means single herb medicine No.5(5) derived from Aeginetia indica that was water extracted (E), acid precipitated (AP) and purified once (1X) by re-dissolution in neutral or alkaline solution and re-precipitation with acid to result in the final chemical entity designated No.5(5)E-APIX.

For example, No.5(5)E-AP1X-NH.sub.4 was prepared by first washing 4.0 to 4.9 g No.5(5)E-AP1X in 50-mL centrifuge tubes, respectively, with about 40 mL water four times. The water washes were separated by centrifuge at 2,000 rpm for 40 minutes and discarded. The water washed AP1X's were freeze dried (total 11.4 g) and then dissolved in about 600 mL 0.1 to 0.2 N NH.sub.4 HCO.sub.3. The solution was centrifuged at 2,000 rpm for 40 minutes and the supernate was filtered through a 0.45-.mu.m filter under vacuum. The filtrate was freeze dried and thus was prepared No.5(5)E-AP1X-NH.sub.4.

GE-AP2X-NH.sub.4 was prepared by dissolving 690.8 mg GE-AP2X in 20.0 mL 0.2 N NH.sub.4 HCO.sub.3. The solution was centrifuged at 8,000 rpm for 40 minutes. No precipitate was observed. The supernate was filtered through a 0.22-.mu.m filter. The filtrate was freeze dried and thus was prepared GE-AP2X-NH.sub.4.

The other samples were prepared in similar fashion.

EXAMPLE 11

Characterization of Active Components

1. No.5(5) Active Components

No.5(5)E-A-AP and No.5(5)E-C-AP were previously identified to be the active components of No.5(5). It appears that these compounds are homologous polymeric organic acids based on their solubilities, HPSEC, C18-SPE-LC, and elemental analyses. Both acids have similar properties, except the molecular weight distribution and retention on C18 column. Both acids are insoluble in acid aqueous solutions but become soluble as salts in neutral and basic aqueous solutions. They are stable to air, heat, acid, weak alkali, and common organic solvents.

Elemental analysis of a six times purified acid precipitate No.5(5)E-AP6X as shown in Table 14 shows a high carbon content (50.98%) and a low ash content (2.35%). This indicates that No.5(5)E-AP6X is an organic acid. The SEM (Scanning Electron Microscopy) x-ray surface elemental analysis of No.5(5)E-AP6X indicated the presence of carbon, oxygen, phosphorus, chlorine, and sulfur. No arsenic, lead, mercury or iron was detected. No.5(5)E-AP6X and its components No.5(5)E-A-AP and No.5(5)E-C-AP, however, are all insoluble in common organic solvents, including ethanol, isopropanol, acetone, acetonitrile, chloroform, and hexane. No.5(5)E-A-AP is also insoluble in methanol. This indicates that

these active acid precipitates are not simple organic acids.

TABLE 14

Elemental Analysis And Ash Contents of Six Times Purified Acid Precipitates (AP6X) of No.5(5)E, GE and HE

Sample	% C	% H	% N	% S	% Cl	% P	% Ash
No.5(5)E-AP6X	50.98	4.92	3.69	0.14	4.69	<0.05	2.35
GE-AP6X	46.47	5.32	5.19	1.21	4.80	0.98	0.60
HE-AP6X	54.69	5.20	3.52	0.66	4.22	1.18	1.99

All acid precipitates (AP) investigated are slightly soluble in water at a slow dissolution rate. All become more soluble and at a more rapid rate in a mixture of water and ethanol, such as water to ethanol at a ratio of 40 to 60 by volume. This indicates that the acid precipitates are of polymeric nature as supported by the HPSEC analysis.

TABLE 15

Anti-HIV Activities And % Weight Distribution Of HPSEC Fractions Of No. 5(5)E-A-APIX And No. 5(5)E-C-AP

HPSEC Fraction	% Weight	Test Level Anti-HIV Activity* (mg/mL)***	Toxicity** Day 3	Day 4
No. 5(5)E-A-AP1X	100%	0.31	98%	75%	87%
No. 5(5)E-A-AP1X-F6	8.3%	0.03	100%	19%	11%
No. 5(5)E-A-AP1X-F7	24.8%	0.08	93%	79%	78%
No. 5(5)E-A-AP1X-F8	33.1%	0.1	94%	80%	83%
No. 5(5)E-A-AP1X-F9	24.8%	0.08	100%	73%	69%
No. 5(5)E-A-AP1X-F10	16.5%	0.05	100%	37%	14%
No. 5(5)E-A-AP1X-F11 to F12	8.3%	0.03	100%	13%	21%
No. 5(5)E-A-AP1X-F13 to F16	8.3%	0.03	96%	9%	15%
No. 5(5)E-C-AP-F7	8.3%	0.03	100%	0%	0%
No. 5(5)E-C-AP-F8	16.7%				

		0.05	100%	95% 85%
No. 5(5)E-C-AP-F9				
	16.7%			
		0.05	100%	98% 92%
No. 5(5)E-C-AP-F10				
	25.0%			
		0.08	100%	80% 35%
No. 5(5)E-C-AP-F1 1 to F12				
	16.7%			
		0.05	100%	59% 8%
No. 5(5)E-C-AP-F13 to F16				
	16.7%			
		0.05	100%	50% 0%

*Activity in % suppression of HIV proliferation based on viral protein p2 level. AntiHIV activity data of No. 5(5)EA-AP1X were the repeated results using solutions stored frozen for three months.
**Toxicity in % of control proliferation.
***Test levels for the HPSEC fractions were equivalent to 0.30 mg/mL of the starting material.

The results clearly indicate that the main activity of No.5(5)E-A-AP1X spreads over three fractions, Fractions 7 to 9, and appears to peak at the mass peak Fraction 8 (80% suppression on day 3 and 83% suppression on day 4 at 0.1 mg/mL). The main activity of No.5(5)E-C-AP also spreads over three fractions, Fractions 8 to 10, and appears to peak at Fractions 8 and 9 (95 to 98% suppression on day 3 and 85 to 92% suppression on day 4 at 0.05 mg/mL. It should be noted that the mass of No.5(5)E-C-AP peaked at Fraction 10 which, however, was only marginally active, 80% suppression on day 3 and 35% suppression on day 4 at 0.08 mg/mL.

One HPSEC fraction, Fraction 8, of No.5(5)E-C-AP was found by ultrafiltration to contain oligomeric molecules of molecular weight between 1,000 and 3,000 dalton. Another HPSEC fraction, Fraction 9, was found to contain polymeric molecules of molecular weight greater than 3,000 dalton. Fraction 9 was more lipophilic than Fraction 8, because Fraction 9 contained larger molecules but eluted later than Fraction 8 on the HPSEC using 0.1 N NH.sub.4 HCO.sub.3 as the mobile phase. Both were tested comparably active: 95% suppression on day 3 and 85% suppression on day 4 for Fraction 8 and 91 to 98% suppression on day 3 and 87 to 92% suppression on day 4 for Fraction 9 at 0.05 to 0.25 mg/mL. Furthermore, a twice chromatographically purified HPSEC Fraction 8 of No.5(5)E-C-AP exhibited a dose response against HIV proliferation and had an IC.sub.50 (50% inhibition concentration) of 6 1 .mu.g/mL on day 3 and 17 .mu.g/mL on day 4. The results are shown in Table 16. As a comparison, the IC.sub.50 of AZT was 3 ng/mL on day 3 and 21 ng/mL on day 4, and that of d4T was 32 nM on day 3 and 540 nM on day 4, when tested concurrently.

TABLE 16

Anti-HIV Activities Of HPSEC Fractions 8 And 9 Of No. 5(5)E-C-AP At Various Concentrations

HPSEC Fraction		Test Level		Anti-HIV Activity*	
	% Wt	(mg/mL)	Toxicity**		
				Day 3	Day 4
Fraction 8 (Run 1)					
	16.7%	0.05	100%	95%	85%

Double chromatographically Purified Fraction 8	10.3%	1.0	100%	93%	85%
		0.3	100%	85%	69%
(Run 2)		0.1	100%	87%	79%
		0.03	100%	89%	81%
		0.01	100%	74%	32%
		0.003	100%	19%	0%
		0.001	91%	3%	5%
Fraction 9 (Run 1)	16.7%	0.05	100%	98%	92%
Fraction 9 (Run 2)	23.6%	0.25	80-82%	91%	87%

*Activity in % suppression of HIV proliferation based on viral protein p2 level.
**Toxicity in % of control proliferation.

The melting points, if there are any, of No.5(5)E-APIX-NH.sub.4 and its purified HPSEC Fraction 8 were determined to be higher than 400.degree. C. This is highly unusual as most organic compounds have melting points of less than 300.degree. C.

2. G and H Active Components

The active components of plants G and H behave similarly to that of No.5(5). Both are polymeric organic acids which are soluble in neutral and slightly basic aqueous solutions and precipitable by acid. Elemental analysis (Table 14) of six times purified acid precipitates of GE and HE shows that both contain high carbon contents (46.47 to 54.69%) and low ash contents (0.60 to 1.99%).

The molecular weights of the water extractable and acid precipitable active components of No.5(5), G and H were estimated by HPSEC analysis to be mostly between 1,000 and 12,000 dalton and, however, some were lower than 1,000 dalton.

3. No.4(2), No.4(3), No.4(4), No.4(5), No.5(1), No.5(8) and No.5(11) Active Components

The active components of all samples tested active were soluble in the neutral cell culture medium. The active components of No.4(2), No.4(5), No.5(1), No.5(8) and No.5(11) are precipitable by acid, while that of No.4(3) is not. The active component of No.4(3) is soluble in both water and acid solution. The active component of No.4(4) is also soluble in water. However, part of the active component of No.4(4) is precipitable by acid and part is not.

The melting point, if there is any, of No.5(8)E-AP1X-NH.sub.4 is higher than 400.degree. C.

4. Anti-HIV Activity Dose Response And Toxicity

As described above, a twice chromatographically purified HPSEC Fraction 8 of No.5(5)E-C-AP exhibits a dose response activity against HIV proliferation as shown in Table 16 and has an IC.sub.50 of 6 .mu.g/mL on day 3 and 17 .mu.g/mL on day 4. Table 17 shows the anti-HIV activity dose responses of the water extractable and acid precipitable active components No.5(5)E-AP1X-NH.sub.4, GE-AP2X-NH.sub.4, HE-AP1X-NH.sub.4, No.5(8)E-AP1X-NH.sub.4, No.5(11)E-AP1X-NH.sub.4 and No.4(2)E-AP1X-NH.sub.4 in their ammonium salt forms. The dose responses of AZT from two different runs are

listed for comparison.

GE-AP2X-NH.sub.4 was not tested at 0.5 mg/mL because of high cytotoxicity (35% of control proliferation) at this concentration. Its cytotoxicity persisted at lower levels: 34% of control proliferation at 0.25 mg/mL and 41% at 0.13 mg/mL. No.4(2)E-AP1X-NH.sub.4 also exhibited high cytotoxicity (44% of control proliferation) at 0.5 mg/mL, but became much less cytotoxic at lower levels: 72% of control proliferation at 0.25 mg/mL and 100% (not cytotoxic) at 0.13 mg/mL.

No.5(5)E-AP1X-NH.sub.4 is cytotoxic towards human PBLs in vitro at 1 mg/mL or higher: 55% of control proliferation at 1 mg/mL, 46% at 2 mg/mL, 11% at 5 mg/mL, and 0% at 10 and 20 mg/mL. No.5 (5)E-AP6X exhibits a slight cytotoxicity (3% of control proliferation) at 0.5 mg/mL and are not toxic at 0.1 mg/mL and lower levels (98% of control proliferation at 0.1 mg/mL and 100% at 0.02 mg/mL). One fraction of the active component of No.5(5)E-AP, i.e., the chromatographically purified HPSEC Fraction 8 of No.5(5)E-C-AP, has been shown to be not cytotoxic: 100% of control proliferation even at 1.0 mg/mL (see Table 16).

The acute toxicity of No.5(5)E-AP1X-NH.sub.4 was investigated. Mice were used to determine acute toxicity and the compound was found not toxic even at a dose of 5,000 mg of the test substance per kilogram of the body weight (fed orally--bolus administration). Four groups of ten (10) male ICR mice (weighing 18 to 21 grams each) per group were used for the acute toxicity test. None of the forty mice were dead seventy two (72) hours after oral administration. The LD.sub.50 of No.5(5)E-AP1X-NH.sub.4 is therefore greater than 5,000 mg/kg (po, mice, 72 hours). Furthermore, tests for effects of No.5(5)E-APIX-NH.sub.4 at 5,000 mg/kg on the central nervous system such as reflex depression, behavior depression, muscle relaxation, motor stimulation and autonomic nervous system of the test animals were all negative when observed one (1) hour and three (3) hours after the oral administration.

TABLE 17

Anti-HIV Activity Dose Responses Of The Water Extractable And Acid Precipitable Active Components Of No. 5(5), No. 5(8), No. 5(11), No. 4(2), G and H In Their Ammonium Salt Forms

Active Component	Test Level (mg/mL)	Anti-HIV Activity* Toxicity** Day 3	Day 4	IC.sub.50
No. 5(5)E-AP1X-NH.sub.4				
	0.5	>55%	98%	95%
	0.05		81%	75%
	0.005		53%	13%
	0.0005		1%	0%
GE-AP2X-NH.sub.4				
	0.05	>41%	100%	96%
	0.005		40%	8%
	0.0005		6%	0%
	0.00005		3%	0%
HE-AP1X-NH.sub.4				
	0.5	100%	92%	95%
	0.05	>89%	87%	95%
	0.005		33%	23%
	0.0005		4%	0%
No. 5(8)E-AP1X-NH.sub.4				

				4.2 .mu.g/mL
				(day 3)
				16 .mu.g/mL
				(day 4)
				7.2 .mu.g/mL
				(day 3)
				20 .mu.g/mL
				(day 4)
				8.7 .mu.g/mL
				(day 3)
				8.3 .mu.g/mL
				(day 4)

	0.5	77%	99%	100%	9.5 .mu.g/mL
	0.05	100%	91%	92%	(day 3)
	0.005		30%	16%	10 .mu.g/mL
	0.0005		0%	0%	(day 4)
No. 5(11)E-AP1X-NH.sub.4					
	0.5	78%	98%	99%	11 .mu.g/mL
	0.05	>99%	91%	92%	(day 3)
	0.005		26%	11%	13 .mu.g/mL
	0.0005		12%	0%	(day 4)
No. 4(2)E-AP1X-NH.sub.4					
	0.5	44%	100%	100%	14 .mu.g/mL
	0.05	100%	88%	83%	(day 3)
	0.005		20%	0%	19 .mu.g/mL
	0.0005		2%	0%	(day 4)
AZT	0.1 .mu.g/mL				
		--	99-100%		
				98-100%	
					2.0 ng/mL
	0.01 .mu.g/mL				
			87-93%		
				84%	(day 3)
	0.001 .mu.g/mL				
			23-53%		
				0-26%	
					4.2 ng/mL
	0.0001 .mu.g/mL				
			3-19%		
				0%	(day 4)

*Activity in % suppression of HIV proliferation based on viral protein p2 level.
**Toxicity in % of control proliferation.

5. Stability of Active Components

The water extractable and acid precipitable active components of No.5(5), No.5(8), No.5(11), No.4(2), G and H are stable to heat, air, strong acids and weak bases such as HCl and ammonium bicarbonate, ammonium hydroxide or sodium bicarbonate, and alcohols such as ethanol. They are active in either acid forms or salt forms such as ammonium or sodium salts.

No.5(5)E-AP1X-NH.sub.4 remained very active (94% suppression on day 3 and 87% suppression on day 4 at 0.1 mg/mL) when tested after 15.6 months of storage at ambient temperature. GE-AP2X-NH.sub.4 and HE-AP1X-NH.sub.4 retained their activities (100% suppression on both day 3 and day 4 for GE-AP2X-NH.sub.4 and 83% suppression on day 3 and 81% suppression on day 4 for HE-AP1X-NH.sub.4 at 0.1 mg/mL) when tested after 13 months of storage at ambient temperature. No.5(8)E-AP1X-NH.sub.4 and No.4(2)E-AP1X-NH.sub.4 remained fairly active (85% suppression on day 3 and 81% suppression on day 4 for No.5(8)E-AP1X-NH.sub.4 and 92% suppression on day 3 and 88% suppression on day 4 for No.4(2)E-AP1X-NH.sub.4 at 0.1 mg/mL) when tested after 12.5 months of storage at ambient temperature. No.5(11)E-AP1X-NH.sub.4 also remained fairly active (84% suppression on day 3 and 78% suppression on day 4 at 0.1 mg/mL) when tested after 12 months of storage at ambient temperature.

INDUSTRIAL APPLICABILITY

The instant invention is directed in part, to the discovery that specific medicinal plants or herbal medicines or their mixtures possess surprising antiviral activities without causing damage to the host cells. Further, the invention is directed to methods of treating humans and mammals infected with viruses such as HBV, HCV, or HIV. The data presented in this application clearly demonstrate that the identified compositions possess antiviral activity without toxicity to the host cells.

It can be concluded from the foregoing experiments that the herb mixture designated HHT888-4 is effective in treating HBV carriers and thus can be used to treat humans infected with HBV. The reduction of viral load in HBV patients and carriers will thus result in the prevention of HBV disease in the human and will also be effective in the treatment of humans exhibiting HBV disease. The clinical tests have also shown that the herb mixture HHT888-45 is effective in treating hepatitis C patients, and thus is expected to be effective in treating hepatitis B patients when administered alone or in combination with HHT888-5 or its antiviral single-herb components.

In addition, HHT888-5, HHT888-45, HHT888-54 and the individual anti-HIV active single-herb components have demonstrated efficacy in suppressing HIV proliferation in native human cells. Furthermore, HHT888-5, HHT888-45 and HHT888-54 have shown efficacy in treating patients infected with HBV and HCV. HHT888-4, HHT888-5, HHT888-45, HHT888-54, water extracts and active principles are also effective in treating humans infected with HIV, including HIV carriers and AIDS patients.

The therapeutic effects described herein may be accomplished through the administration of the herbal medicines "as is", or as teas, decoctions, beverages, candies or other confections, enteral liquid nutritional products such as infant formula and adult nutritional products, medical foods, nutritional supplements or neutraceuticals containing one or more of the herbal medicines or their extracts or the active principles. For pharmaceutical preparations, one or more of the antiviral herbal medicines or their extracts or active principles described above may be administered in unit dosage forms such as capsules, packets or tablets, with or without controlled-release coating(s).

The medical community is constantly in search of methods and products that will effectively treat viral infections, especially methods and products for treating humans infected with HBV, HCV, and HIV. The herb mixtures HHT888-4, HHT888-5, HHT888-45, HHT888-54, the single-herb components, their extracts, active principles and products containing these herbal compositions will be readily accepted by the medical community as an additional tool in the prevention and treatment of these devastating illnesses.

While certain representative embodiments have been described herein, it will be apparent to those skilled in the art that various changes and modifications may be made therein without departing from the spirit or scope of this invention.

* * * * *

LEVEL 1 - 7 OF 7 PATENTS

348,509

<=2> GET EXEMPLARY DRAWING

January 3, 1990

ANTI-RETROVIRAL DRUG

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ENCH-TITLE: MEDICAMENT ANTIRETROVIRAL

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UM:

The present invention relates to an anti-retroviral agent effective for the nedy of viral diseases caused by human retroviruses.

Vaccination has generally been adopted as a means of preventing infection :h a virus, and with the development of the scientific techniques, agents for

Vaccination has generally been adopted as a means of preventing infection ith a virus, and with the development of the scientific techniques, agents for ontrolling the propagation of certain viruses have been developed.

Among various viruses, HIV (human immunodeficiency virus) causing an acquired mmune deficiency syndrome (AIDS) and HTLV-1 (human T-cell leukemia virus type causing human T-cell leukemia are known as retroviruses.

A retrovirus is a virus containing an RNA-dependent DNA synthesis enzyme ıereinafter referred to as "reverse transcriptase") in virus particles, and ıis virus grows in the following manner.

(1) After the virus infects a host cell, viral RNA is transcribed to viral IA by the action of reverse transcriptase.

(2) Viral DNA is integrated in the chromosomal DNA of the host cell, and .ral mRNA is then synthesized by a cellular RNA polymerase of the host cell.

(3) Viral proteins are produced by viral mRNA.

(4) The proteins formed by the above-mentioned mRNA bond to genome RNA to ırm a filial virus, and this filial virus leaves the cell.

A medicinal agent having an epoch-making curative effect to human diseases used by retroviruses has not been developed, and the development of such a dicinal agent is urgently required.

Archives of Virology, 57,(1978) pp 255-260 and Chem. Abstracts 102,(1985)pp 0, 102:92800m disclose that some flavonoids including quercetin and hesperidin ve anti-viral effects against certain viruses (none of which are troviruses).

Antiviral Research, 7,(1987) pp 127-137 discloses an action of ethylquercetin on poliovirus.

Experientia, 36,(1980) pp 304 discloses that glycyrrhizic acid inhibits the owth of several DNA and RNA viruses in cell cultures and inactivates Herpes mplex 1 virus irreversibly.

A primary object of the present invention is to provide an anti-retroviral ent effective for the remedy of human diseases caused by retroviruses.

The present inventors investigated the anti-retroviral effect of various rbal drugs and plant components, and as a result, found that

The present inventors investigated the anti-retroviral effect of various rbal drugs and plant components, and as a result, found that ao-Chai-Hu-Tang, which is a Chinese medicine preparation comprising Bupleuri adix, Scutellariae radix, Glycyrrhizae radix, Ginseng radix, Zingiberis izoma, Zizyphi fructus and Pinelliae tuber, baicalein and baicalin extracted d isolated from a herb drug Scutellariae radix, quercetin extracted and lated from a herb drug Sophora japonica, hesperetin extracted and isolated m a herb drug Aurantii nobilis pericarpium, and alizarin ,2-dihydroxyanthraquinone), which is a red pigment of madder, a plant longing to the family Rubia, and a derivative thereof, have an anti-retroviral fect, and the present invention was completed based on this finding.

More specifically, in accordance with the present invention, there is ovided the use in manufacturing an anti-retroviral agent of, as an effective gredient, at least one member selected from Xiao-Chai-Hu-Tang, baicalein, icalin, quercetin, hesperetin, and alizarin and derivatives and armacologically acceptable salts thereof, represented by the following formula (collectively referred to as "compound of formula I" hereinafter): wherein R, and R, which may be the same or different, stand for a hydrogen atom or a droxyl group, and R stands for a hydrogen atom, a sulfonic acid group or a carboxymethylaminomethyl group.

The constituent herb drugs, doses and extraction methods of Xiao-Chai-Hu-Tang e described in classical literature references concerning Chinese medicine eparations (such as Shang-Han-Lun and Jin-Kui-Yao-Lue), and this preparation s been used for the remedy of liver troubles, chronic gastroenteric troubles, stpartal recovery deficiency, and other diseases, but it has not been known at Xiao-Chai-Hu-Tang has an anti-retroviral effect.

Any of the preparations of Xiao-Chai-Hu-Tang formed according to recipes of rb drugs disclosed in classic literature references such as Shang-Han-Lun and n-Kui-Yao-Lue can be used as Xiao-Chai-Hu-Tang in the present invention.

With regard to the mixing ratios of the respective herb drugs, ao-Chai-Hu-Tang preferably comprises 4 to 7 parts by weight of Bupleuri radix, parts by weight of Scutellariae radix, 2 parts by weight of Glycyrrhizae dix, 2 to 3 parts by weight of Ginseng radix, 1 part by weight of Zingiberis izoma, 2 to 3 parts by weight of Zizyphi fructus, and 4 to 5 parts by weight Pinelliae tuber.

Xiao-Chai-Hu-Tang, which is formed, for example, by boiling 7 g of Bupleuri dix, 3 g of Scutellariae radix, 2 g of Glycyrrhizae radix, 3 g of Ginseng dix, 1 g Zingiberis rhizoma, 3 g of Zizyphi fructus and 5 g of Pinelliae ber in 600 ml of water until the volume is reduced to 350 ml, removing the

uber in 600 ml of water until the volume is reduced to 350 ml, removing the regs, and making a decoction of only the medicinal liquid until the volume is educed to 200 ml, can be divided into three dosages, to be taken three times er day, but a dry extract powder or a Chinese herb extract medicine formed from he above liquid extract can be used as the anti-retroviral agent in view of ase of administration and portability thereof.

In view of the pharmacological effect desired preferably the iao-Chai-Hu-Tang prepared by the following method is used.

More specifically, according to the teachings of Shang-Han-Lun and in-Kui-Yao-Lue, 7 g of Bupleuri radix, 3 g of Scutellariae radix, 2 g of lycyrrhizae radix, 3 g of Ginseng radix, 1 g of Zinziberis rhizoma, 3 g of izyphi fructus, and 5 g of Pinelliae tuber are mixed with distilled water in an nount of 10 to 12 times the amount of the herb drug mixture, extraction is arried out at 95 to 100C for about 60 minutes, solid-liquid separation is arried out, and the obtained liquid is spray-dried to obtain a dry extract owder of Xiao-Chai-Hu-Tang (4.5 g of the dry extract powder contains 25.0 to 2.0 mg of glycyrrhizin, 90 to 210 mg of baicalin, and 2.3 to 6.9 mg of aikosaponin b2).

For the formulation, an excipient, an adjuvant, and other additives istomarily used in the pharmaceutical field are added to the dry extract owder, and the mixture is formed into a powder, a granule, a tablet or a apsule, by methods customarily used in the pharmaceutical field.

A specific example of an anti-retroviral agent comprising Xiao-Chai-Hu-Tang the effective ingredient will now be described.
ecific Example

To 7 g of Bupleuri radix, 3 g of Scutellariae radix, 2 g of Glycyrrhizae dix, 3 g of Ginseng radix, 1 g of Zingiberis rhizoma, 3 g of Zizyphi fructus, d 5 g of Pinelliae tuber was added 300 ml of distilled water, and extraction carried out at 100C for 60 minutes. A solid-liquid separation was effected centrifugal separation, and the supernatant was spray-dried below 50C to tain a dry extract powder of Xian-Chai-Hu-Tang. When the amounts of the mponents contained in 4.5 g of the dry extract powder were determined, it was und that 42.5 mg of glycyrrhizin, 160 mg of baicalin, and 4.5 mg of ikosaponin b2 were contained.

It is known that baicalein and baicalin have an anti-inflammatory action, an ti-allergic action, and an anti-hypertensive action, but it has not been known at these compounds have an anti-viral action, especially an anti-retro-viral tion.

:tion.

It is also known that quercetin and hesperetin have an anti-inflammatory :tion, an anti-allergic action, and an anti-hypertensive action, and that these mpounds control the growth of a DNA virus, but it has not been known that ese compounds have an anti-retroviral effect on a retrovirus which has a erse transcriptase and grows through this reverse transcriptase.

Baicalin and baicalein are compounds represented by the following chemical ructural formula II and are marketed by Wako Junyaku Kabushiki Kaisha: wherein R stands for H, the compound is baicalein and if R stands for CHO(glucuronic id residue), the compound is baicalin.

Quercetin is a compound represented by the following chemical structural rmula III and is marketed by Tokyo Kasei Kogyo Kabushiki Kaisha: Hesperetin is compound represented by the following chemical structural formula IV and is rketed by Tokyo Kasei Kogyo Kabushiki Kaisha: The compound of formula I used the present invention is known as a pigment, but it has not been known that e compound has an anti-viral effect, especially an anti-retroviral effect.

As the compound of formula I, alizarin that can be easily extracted and olated from madder, a plant belonging to the family rubia, can be directly ed. Derivatives of alizarin can be easily prepared from this alizarin, and rivatives marketed as reagents also can be used as the compound of formula I.

Specific examples of the compound of formula I are shown below.

With reference to the following experiments, the anti-retroviral effect of e anti-retroviral agent of the present invention will now be explained.

periment 1

Cells infected with mouse leukemia virus were cultured, and the reverse anscriptase was separated and purified by the method of Nakajima et al. PARATIVE LEUKEMIA RESEARCH 1973, LEUKEMOGENESIS, ED. Y. ITO AND R.M. DUTCHER, IV. OF TOKYO PRESS TOKYO/KARGER, BASEL, pp. 603-605 (1975)!, and then a action mixture liquid having the following composition was prepared.

verse transcriptase	1 unit/m 1
yadenylic acid / oligothymidylic acid duplex polyadenylic d (supplied by Pharmacia / oligothymidylic acid (supplied Pharmacia) = base ratio 4/1! as temperate / primer	2 Ng/ml
s-hydrochloric acid (pH 8.0)	50 mM

is-hydrochloric acid (pH 8.0)	50 mM
thiothreitol	5 mM
tassium chloride	50 mM
nganese chloride	0.2 mM
-Deoxythymidine triphosphate (referred to as "H!dTTP reinafter!	0.01 mM (400 cpm/pm ol)
ycerol	15 (v/v)%
stilled water	appropr iate amount

te

By "1 unit" is meant the unit of the relative activity of the reverse anscriptase required to consume 1 nmol of dNTP (deoxynucleic acid phosphate at C for 1 hour.

Then the dry extract powder obtained in the above-mentioned Specific Example, icalein, baicalin, quercetin or hesperetin and refined water were added to 20 of the above-mentioned reaction mixture liquid so that the total volume was Nl, the incorporation of the radioactivity of H!dTTP into the acid-insoluble action was measured by a Beckmann scintillation counter, and the percent hibition was calculated as the reverse transcriptase activity with respect to ch concentration. The results are shown in Tables 1 through 4.

Table 1

	0.53
	12.02
	19.25
	37.32
)	84.51
)	93.5
)	99.5

Table 2

l	29	-
2	35	-
3	74	-

.5 74 -
92 19
98 27
100 73
) 100 90
) 100 100

ble 3

77
96
99
) 100

) 100

) 100

ble 4

99
100

100

100

100

100

periment 2

MT-2 cells were cultured at a concentration of 3 x 104 cells/ml in a culture dium of RPMI 1640 (supplied by Gibco Co.) containing 10% FCS (supplied by bco Co.). After 2 days, the MT-2 cells were infected with HIV at a ratio of 0 cells per HIV, were distributed into groups to which 5 Ng/ml of baicalein, ercetin or hesperetin was added and non-added groups (control groups), and the fect of the addition was determined. After 1 week, the number of living cells s counted, and it was confirmed that the modification of MT-2 cells with HIV s inhibited at a ratio of at least 90% .

periment 3

A reaction mixture liquid having the following composition was prepared.

A reaction mixture liquid having the following composition was prepared.

verse transcriptase (Rous Associated Virus-2 supplied by kara Shuzo)	2 units /ml
lyadenylic acid/oligothymidylic acid duplex polyadenylic acid alyrA) (supplied by Pharmacia), oligothymidylic acid (dT) pplied by Pharmacia)! as temperate/primer	10 Ng/ml
is-hydrochloric acid (pH 8.3)	50 mM
tassium chloride	50 mM
gnesium chloride	10 mM
dTTP (40 ci/mmol) (1.0 mCi/ml)	0.2 NM
oxythymidine triphosphate	9.8 NM
stilled water	approp riate amoun t

A reaction of 50 Nl of the reaction mixture liquid was carried out at 37C for minutes. The reaction product DNA was determined by the ion exchange filter per method, the incorporation of the radioactivity ofH!dTTP into DNA was asured by a Beckmann scintillation counter, and the 50% inhibition ncentration (IC) of each test substance was calculated as the reverse anscriptase activity. The results are shown in Table 5.

Table 5

zarin	12.5
zarin complexone	3.8
zarine Red S	7.0
nalizarin	7.7
purin	17.5

periment 4

A 24-well microplate was charged with 3.5 x 105cells per well of primary cken embryo fibroblasts, and the fibroblasts were cultured at 37C in an osopher of 5% CO for 6 hours. Then an RSV. SR-A strain having an infection tiplicity (M.O.I.) of about 2.0 x 104 and the test substance were added, and turing was continued for further 2 hours. The cells were washed and the test stance and an agar culture medium were added, and then culturing was tinued at 37C in an atmospher of 5% CO for further 8 days. The number of foci

:ontinued at 37C in an atmospher of 5% CO for further 8 days. The number of foci ras counted, and the results are shown as IC in Table 6.

Table 6

.lizarin complexon	4.3
.zarine Red S	2.1

From the results of Experiments 1 through 4, it was confirmed that the nti-retroviral agent of the present invention has an anti-retroviral effect ased on the action of inhibiting the reverse transcriptase activity.

Namely, the anti-retroviral agent of the present invention controls the rowth of the retrovirus by inhibiting the reverse transcriptase activity ecessary for the growth of the retrovirus. Accordingly, the agent of the resent invention can be applied to any retrovirus.

As specific examples of the retroviruses, there can be mentioned leukemia irus, sarcoma virus, mammary tumor virus, visna virus, maedic virus, HIV and TLV-1.

The compound of formula I has not only an action of inhibiting the reverse ranscriptase activity but also an action of controlling the etrovirus-producing activity of retrovirus-infected cells.

An experiment demonstrating this action of the retrovirus-producing activity ill now be described.

xperiment 5

The test substance and 1 x 105 cells per well of MT-2 cells were charged in a -well microplate, and culturing was conducted for 4 days. Then the MT-2 cell spension in each well was subjected to centrifugal separation, and the ernatant was extracted with phenol to obtain RNA of HTLV-1. This RNA and a own amount of HTLV-1 cDNA were plotted on a nitrocellulose paper, a mparative determination was carried out according to the hybridization using labelled HTLV-1 cDNA, and the hydridization rate by the test substance was lculated by comparing the amount of the produced virus with that of the ntrol.

The results are shown as the hybridization rate (%) in Table 7.

Table 7

izarine Red S 20 61.7
rpurin 5 61.3

From the results of Experiment 5, it was confirmed that the compound of rmula I has an effect of controlling the retrovirus-producing activity of rovirus-infected cells.

When the acute toxicity of the anti-retroviral agent of the present invention mprising Xiao-Chai-Hu-Tang by oral administration was tested by using male ce of the ddY line and male rats of the Wistar line, it was found that no imal died even if the anti-retroviral agent of the present invention obtained the above-mentioned Specific Example was orally administered at a dose of 15 g (administration limit).

As seen from this result, the anti-retroviral agent of the present invention mprising Xiao-Chai-Hu-Tang has a very low toxicity and a high safety. Note, ao-Chai-Hu-Tang has been clinically used as a Chinese herb medicine eparation for many decades, and it has been confirmed that this preparation substantially no adverse side effects.

When the acute toxicity test of baicalin, baicalein, quercetin, and peretin was carried out by using male mice of the ddY line and male rats of Wistar line, it was found that no animal died by oral administration at a e of 1 g/kg. As apparent from this result, baicalin, baicalein, quercetin, hesperetin have a very low toxicity and a high safety.

It is known that the LD values of alizarin and Alizarine Red S upon ravenous administration are 120 mg/kg and 70 mg/kg, respectively, and ordingly, it is++1999288907791obvious that the compound of formula I also has ery low toxicity and a high safety.

In view of the experimental data of the present invention and the acute icity test results, it is considered that the effective administration amount the anti-retroviral agent comprising Xiao-Chai-Hu-Tang is such that 1 to 10 g the agent as the dry extract powder can be divided into three doses and inistered three times per day for an adult, though the effective inistration amount differs to some extent according to the age and body ght of the patient and the seriousness of the disease.

Administration amounts and formulation methods for baicalin, baicalein, rcetin, and hesperetin will now be described.

Baicalin, baicalein, quercetin, hesperetin, and the compound of formula I can

Baicalin, baicalein, quercetin, hesperetin, and the compound of formula I can e administered to animals and humans directly or together with customarily used harmaceutical carriers. The administration form is not particularly critical nd an appropriate administration form is selected according to need. For xample, there can be mentioned oral medicines such as tablets, capsules, ranules, fine granules and powders, and non-oral medicines such as injections suppositories.

The dose of the oral medicine required to obtain the intended effect differs o some extent according to the age and body weight of the patient and the egree of the disease, but preferably 100 to 6000 mg as the weight of baicalin, aicalein, quercetin, hesperetin or the compound of formula I is generally dministered dividedly several times per day for an adult.

In the present invention, oral medicines such a tablets, capsules and ranules are prepared by customary procedures using, for example, starch, actose, refined sugar, mannitol, carboxymethyl cellulose, corn starch, and norganic salts.

For formation of these medicines, binders, disintegrating agents, surface ctive agents, lubricants, flowability improvers, taste improvers, colorants, nd perfumes can be used in addition to the above-mentioned excipients. Specific camples thereof are described below.
Binders)

Starch, dextrin, gum arabic, gelatin, hydroxypropylstarch, methylcellulose, odium carboxymethyl cellulose, hydroxypropyl cellulose, crystalline cellulose, thylcellulose, polyvinyl pyrrolidone, and macrogall.
Disintegrating Agents)

Starch, hydroxypropylstarch, sodium carboxymethyl cellulose, calcium rboxymethyl cellulose, carboxymethyl cellulose, and lower substituted droxypropyl cellulose.
rface Active Agents)

Sodium lauryl sulfate, soybean lecithin, sucrose, fatty acid ester, aluminum earate, and Polysolvate 80.
ubricants)

Talc, wax, hydrogenated vegetable oil, sucrose fatty acid ester, magnesium earate, calcium stearate, aluminum stearate, and polyethylene glycol.
lowability Improvers)

Light silicic anhydride, dry aluminum hydroxide gel, synthetic aluminum

Light silicic anhydride, dry aluminum hydroxide gel, synthetic aluminum
licate, and magnesium silicate.

Baicalin, baicalein, quercetin, hesperetin, and the compound of formula I can
administered in the form of suspensions, emulsions, syrups, and elixirs.
ese preparations may further contain a taste improver, a smell improver, and a
orant.

The dosage of the non-oral medicine for obtaining the intended effect varies
some extent depending on the age and body weight of the patient and the
gree of the disease, but preferably is administered as 1 to 100 mg as the
ight of baicalein, baicalin, quercetin, hesperetin or the compound of formula
er day for an adult by intravenous injection, intravenous drip, hypodermic
jection, or intramuscular injection.

This non-oral medicine is prepared by customary procedures. As the diluent,
re can be used distilled water for injection, physiological saline solution,
aqueous solution of glucose, a vegetable oil for injection, sesame oil,
onut oil, soybean oil, corn oil, propylene glycol and polyethylene glycol. A
gicide, an antiseptic agent and a stabilizer can be added according to need.
view of the stability of the non-oral medicine, a method can be adopted in
ch the non-oral medicine is filled in a vial and frozen, water is removed by
conventional freeze-drying technique, and the liquid is prepared again just
ore application. An isotropic agent, a stabilizer, an antiseptic agent, and
analgesic agent can be added according to need.

As other non-oral agents, there can be mentioned coating agents such as
ernal lotions and ointments, and suppositories for intrarectal
inistration. These non-oral medicines can be prepared by customary
cedures.

The anti-retroviral agent of the present invention will now be described in
ail with reference to the following examples, that by no means limit the
pe of the invention.
mple 1

The dry extract powder (200 g) obtained in the Specific Example was mixed
h 89 g of lactose and 1 g of magnesium stearate and the mixture was tableted
a single-shot tableting machine to form slug tablets having a diameter of 20
and a weight of about 2.3 g. The tablets were pulverized by an oscillator,
jected to particle size adjustment, and sieved to obtain a good granule
sisting of particles having a size of 20 to 50 mesh.

This granule was administered at a dose of 0.5 to 4.5 g (corresponding to

This granule was administered at a dose of 0.5 to 4.5 g (corresponding to 34 to 3.10 g of the dry extract powder) three times a day according to the sease conditions.

ample 2

The dry extract powder (200 g) obtained in the Specific Example was mixed h 20 g of microcrystalline cellulose and 5 g of magnesium stearate, and the cure was tableted by a one-shot tableting machine to form tablets having a ameter of 7 mm and a weight of 225 mg. Each tablet contained 200 mg of the dry tract powder of the anti-retroviral agent of the present invention. Two to xteen tablets were administered three times a day according to the disease nditions.

ample 3

The dry extract powder obtained in the Specific Example was filled in hard psules so that each capsule contained 500 mg of the powder. Two to twenty psules were administered three times a day according to the disease nditions.

ample 4

) Corn starch	44 g
) Crystalline cellulose	40 g
) Calcium carboxymethyl cellulose	5 g
) Light silicic anhydride	0.5 g
) Magnesium stearate	0.5 g
) Baicalin	10 g
Total	100 g

According to the above-mentioned recipe, the components (1) through (6) were nogeneously mixed, and the mixture was compression-molded by a tableting chine to obtain tablets, each having a weight of 200 mg.

Each tablet contained 20 mg of baicalin, and 5 to 7 tablets were dividedly ministered several times per day for an adult.

ample 5

Crystalline cellulose	84.5 g
Magnesium stearate	0.5 g
Calcium carboxymethyl cellulose	5 g
Baicalein	10 g
Total	100 g

According to the above-mentioned recipe, the components (1) and (4) and a art of the component (2) were homogeneously mixed, and the mixture was ompression-molded and then pulverized and the component (3) and the remainder : the component (2) were added to the pulverization product. The mixture was ompression-molded by a tableting machine to form tablets, each having a weight : 200 mg.

Each tablet contained 20 mg of baicalein, and 5 to 7 tablets were dividedly dministered several times per day for an adult.

ample 6

) Crystalline cellulose	34.5 g
) 10% Ethanol solution of hydroxypropyl cellulose	50 g
) Calcium carboxymethyl cellulose	5 g
) Magnesium stearate	0.5 g
) Baicalin	10 g
Total	100 g

According to the above-mentioned recipe, the components (1), (2) and (5) were mogeneously mixed, and according to customary procedures, the mixture was eaded, granulated by an extrusion granulator, dried, and disintegrated. Then e disintegration product was mixed with the components (3) and (4) and the xture was compression-molded by a tableting machine to obtain tablets each ving a weight of 200 mg.

Each tablet contained 20 mg of baicalin, and 5 to 7 tablets were dividedly ministered several times per day for an adult.

ample 7

) Corn starch	84 g
) Magnesium stearate	0.5 g
Calcium carboxymethyl cellulose	5 g
) Light silicic anhydride	0.5 g
) Baicalein	10 g
Total	100 g

According to the above-mentioned recipe, the components (1) through (5) were nogeneously mixed, and the mixture was compression-molded by a compression lding machine, pulverized by a pulverizer and sieved to obtain a granule.

One gram of this granule contained 100 mg of baicalein, and 1 to 2.5 g of the anule was dividedly administered several times per day for an adult.

One gram of this granule contained 100 mg of baicalein, and 1 to 2.5 g of the
anule was dividedly administered several times per day for an adult.

ample 8

) Crystalline cellulose	40 g
10% Ethanol solution of hydroxypropyl cellulose	50 g
Baicalin	10 g
Total	100 g

According to the above-mentioned recipe, the components (1) through (3) were
mogeneously mixed and kneaded. The kneaded mixture was granulated by a
anulator, dried, and sieved to obtain a granule.

One gram of this granule contained 100 mg of baicalin, and 1 to 2.5 g of the
anule was dividedly administered several times per day for an adult.

ample 9

) Corn starch	89.5 g
) Light silicic anhydride	0.5 g
) Baicalein	10 g
Total	100 g

According to the above-mentioned recipe, the components (1) through (3) were
mogeneously mixed, and the mixture was filled in capsules No. 2 so that each
psule contained 200 mg of the mixture.

Each capsule contained 20 mg of baicalein, and 5 to 7 capsules were dividedly
ministered several times per day for an adult.

ample 10

Distilled water for injection	appropriate amount
) Glucose	200 mg
) Baicalin	10 mg
Total	15 ml

The components (2) and (3) were dissolved in distilled water for injection,
i the solution was filled in an ampoule having a capacity of 5 ml.
erilization was carried out at 121C for heating under pressure to obtain an
jection.

ample 11

1) Crystalline cellulose	84.5 g
2) Magnesium stearate	0.5 g
3) Calcium carboxymethyl cellulose	5 g
4) Quercetin	10 g
Total	100 g

According to the above-mentioned recipe, the components (1) and (4) and a art of the component (2) were homogeneously mixed and the mixture was ompression-molded and pulverized. Then the component (3) and the remainder of ne component (2) were added to the pulverization product, and the mixture was ompression-molded by a tableting machine to obtain tablets, each having a eight of 200 mg.

Each tablet contained 20 mg of quercetin, and 5 to 7 tablets were dividedly dministered several times per day for an adult.

ample 12

1) Corn starch	84 g
2) Magnesium stearate	0.5 g
3) Calcium carboxymethyl cellulose	5 g
4) Light silicic anhydride	0.5 g
5) Quercetin	10 g
Total	100 g

According to the above-mentioned recipe, the components (1) through (5) were omogeneously mixed and the mixture was compression-molded by a compression olding machine, pulverized by a pulverizer and sieved to obtain a granule.

One gram of this granule contained 100 mg of quercetin, and 1 to 2.5 g of the anule was dividedly administered several times per day for an adult.

ample 13

1) Corn starch	89.5 g
2) Light silicic anhydride	0.5 g
3) Quercetin	10 g
Total	100 g

According to the above-mentioned recipe, the components (1) through (3) were mogeneously mixed and the mixture was filled in capsules No. 2 so that each psule contained 200 mg of the mixture.

Each capsule contained 20 mg of quercetin, and 5 to 7 capsules were dividedly dministered several timer per day for an adult.

xample 14

1)	Distilled water for injection	appropriate amount
	Glucose	200 mg
	Quercetin	10 mg
	Total	15 ml

The components (2) and (3) were dissolved in distilled water for injection nd the solution was filled in an ampoule having a capacity of 5 ml and ubjected to sterilization at 121C for 15 minutes under pressure to obtain an njection.

xample 15

.)	Crystalline cellulose	84.5 g
:)	Magnesium stearate	0.5 g
:)	Calcium carboxymethyl cellulose	5 g
.)	Hesperetin	10 g
	Total	100 g

According to the above-mentioned recipe, the components (1) and (4) and a rt of the component (2) were homogeneously mixed and the mixture was mpression-molded and pulverized. Then the component (3) and the remainder of ie component (2) were added to the pulverization product, and the mixture was mpression-molded by a tableting machine to obtain tablets, each having a ight of 200 mg.

Each tablet contained 20 mg of hesperetin, and 5 to 7 tablets were dividedly ministered several times per day for an adult.

xample 16

)	Corn starch	84 g
)	Magnesium stearate	0.5 g
)	Calcium carboxymethyl cellulose	5 g
)	Light silicic anhydride	0.5 g
)	Hesperetin	10 g
	Total	100 g

According to the above-mentioned recipe, the components (1) through (5) were mogeneously mixed and the mixture was compression-molded by a compression

According to the above-mentioned recipe, the components (1) through (5) were omogeneously mixed and the mixture was compression-molded by a compression olding machine, pulverized by a pulverizer and sieved to obtain a granule.

One gram of this granule contained 100 mg of hesperetin, and 1 to 2.5 g of he granule was dividedly administered several times per day for an adult.

mple 17

1)	Corn starch	89.5 g
2)	Light silicic anhydride	0.5 g
3)	Hesperetin	10 g
	Total	100 g

According to the above-mentioned recipe, the components (1) through (3) were omogeneously mixed and the mixture was filled in capsules No. 2 so that each apsule contained 200 mg of the mixture.

Each capsule contained 20 mg of hesperetin, and 5 to 7 capsules were ividedly administered several times per day for an adult.

kample 18

1)	Distilled water for injection	appropriate amount
2)	Glucose	200 mg
3)	Hesperetin	10 mg
	Total	15 ml

The components (2) and (3) were dissolved in distilled water for injection, nd the solution was filled in an ampoule having a capacity of 5 ml and ubjected to sterilization at 121C for 15 minutes under pressure to obtain an njection.

xample 19

.)	Corn starch	44 g
:)	Crystalline cellulose	40 g
)	Calcium carboxymethyl cellulose	5 g
)	Light silicic anhydride	0.5 g
)	Magnesium stearate	0.5 g
)	Alizarin	10 g
	Total	100 g

According to the above-mentioned recipe, the components (1) through (6) were

According to the above-mentioned recipe, the components (1) through (6) were
›mogeneously mixed, and the mixture was compression-molded by a tableting
ıchine to obtain tablets, each having a weight of 200 mg.

Each tablet contained 20 mg of alizarin, and 5 to 7 tablets were dividedly
lministered several times per day for an adult.

mple 20

) Crystalline cellulose	84.5 g
) Magnesium stearate	0.5 g
) Calcium carboxymethyl cellulose	5 g
) Alizarin complexon	10 g
Total	100 g

According to the above-mentioned recipe, the components (1) and (4) and a
rt of the component (2) were homogeneously mixed, and the mixture was
mpression-molded and pulverized. Then the component (3) and the remainder of
e component (2) were added to the pulverization product, and the mixture was
mpression-molded by a tableting machine to obtain tablets, each having a
ight of 200 mg.

Each tablet contained 20 mg of alizarin complexon, and 5 to 7 tablets were
videdly administered several times per day for an adult.

ample 21

) Crystalline cellulose	34.5 g
) 10% Ethanol solution of hydroxypropyl cellulose	50 g
) Calcium carboxymethyl cellulose	5 g
) Magnesium stearate	0.5 g
) Alizarine Red S	10 g
Total	100 g

According to the above-mentioned recipe, the components (1), (2) and (5) were
nogeneously mixed, and according to customary procedures, the mixture was
eaded, granulated by an extrusion granulator, dried and disintegrated. Then
e components (3) and (4) were added to the disintegration product and the
cture was compression-molded by a tableting machine to obtain tablets each
ving a weight of 200 mg.

Each tablet contained 20 mg of Alizarine Red S, and 5 to 7 tablets were
videdly administered several times per day for an adult.

mple 22

xample 22

1) Corn starch	84 g
2) Magnesium stearate	0.5 g
3) Calcium carboxymethyl cellulose	5 g
Light silicic anhydride	0.5 g
Quinalizarin	10 g
Total	100 g

According to the above-mentioned recipe, the components (1) through (5) were omogeneously mixed, and the mixture was compression-molded by a compression olding machine, pulverized by a pulverizer, and sieved to obtain a granule.

One gram of this granule contained 100 mg of quinalizarin, and 1 to 2.5 g of he granule was dividedly administered several times per day for an adult.

xample 23

1) Crystalline cellulose	55 g
2) 10% Ethanol solution of hydroxypropyl cellulose	35 g
3) Purpurin	10 g
Total	100 g

According to the above-mentioned recipe, the components (1) through (3) were omogeneously mixed, and the mixture was kneaded, granulated by an extrusion ranulator, dried, and sieved to obtain a granule.

One gram of this granule contained 100 mg of purpurin, and 1 to 2.5 g of the ranule was dividedly administered several times per day for an adult.

xample 24

Corn starch	89.5 g
2) Light silicic anhydride	0.5 g
3) Alizarin complexon	10 g
Total	100 g

According to the above-mentioned recipe, the components (1) through (3) were omogeneously mixed, and the mixture was filled in capsules No. 2 so that each apsule contained 200 mg of the mixture.

Each capsule contained 20 mg of alizarin complexon, and 5 to 7 capsules were videdly administered several times per day for an adult.

Each capsule contained 20 mg of alizarin complexon, and 5 to 7 capsules were videdly administered several times per day for an adult.

ample 25

)	Distilled water for injection	appropriate amount
)	Glucose	100 mg
.	Alizarin complexon	10 mg
	Total	15 ml

The components (2) and (3) were dissolved in distilled water for injection, d the solution was filled in an ampoule having a capacity of 5 ml and bjected to sterilization at 121C for 15 minutes under pressure to obtain an jection.

NGLISH-CLAIMS:

1. Use of at least one substance selected from Xiao-Chai-Hu-Tang, baicalein, icalin, quercetin, hesperetin, and alizarin and derivatives and armacologically acceptable salts thereof represented by the following formula wherein R, R and R, which may be the same or different, stand for a hydrogen om or a hydroxyl group, and R stands for a hydrogen atom, a sulfonic acid oup or a dicarboxymethylaminomethyl group, in the manufacture of an ti-retroviral agent.

2. Use according to claim 1 wherein the anti-retroviral agent is manufactured ing Xiao-Chai-Hu-Tang which is prepared from 4 to 7 parts by weight of pleuri radix, 3 parts by weight of Scutellariae radix, 2 parts by weight of ycyrrhizae radix, 2 to 3 parts by weight of Ginseng radix, 1 part by weight of ngiberis rhizoma, 2 to 3 parts by weight of Zizyphi fructus, and 4 to 5 parts weight of Pinelliae tuber.

3. Use according to claim 2 wherein the Xiao-Chai-Hu-Tang is obtained in the rm of a dry extract powder, and 4.5 g of the dry extract powder contains 25.0 52.0 mg of glycyrrhizin, 90 to 210 mg of baicalin and 2.3 to 6.9 mg of ikosaponin b2.

4. Use according to claim 2 wherein the anti-retroviral agent is for ministration to a human adult patient in an amount of from 1 to 10 g per day.

5. Use as set forth in claim 1, wherein the effective ingredient is selected om baicalein, baicalin, quercetin, hesperetin, and compounds of the formula I l salts thereof, and the anti-retroviral agent is administered in an amount of) to 6000 mg as the effective ingredient per day for an adult.

LEVEL 1 - 197 OF 578 PATENTS

568,001

November 3, 1993

Antiviral agent containing crude drug

ERMAN-TITLE: Ein Roharzneimittel enthaltende antivirales Mittel

RENCH-TITLE: Agent antiviral contenant un medicament brut

PPL-NO: 93106801

ILED: April 27, 1993

RIORITY: April 27, 1992, Japan (JP), 107659/92; April 27, 1992, Japan (JP),)7672/92

SSIGNEE: Namba, Tsuneo, 1-104, 2556-4, Gofukusuehiro-cho, Toyama-shi, Toyama apan (JP); DESIGNATED STATES: Europe (EP); Afghanistan (AF); Albania (AL); .geria (DZ); Andorra (AD); Angola (AO); Anguilla (AI); Antigua (AG); :gentina (AR); Armenia (AM); Australia (AU); Austria (AT); Azerbaijan (AZ); hamas (BS); Bahrain (BH); Bangladesh (BD); Barbados (BB); Belarus (BY); :lgium (BE); Belize (BZ); Benin (BJ); Bermuda (BM); Bhutan (BT); Bolivia (BO);)tswana (BW); Brazil (BR); British Virgin Islands (VG); Brunei (BN); Bulgaria G); Burkina Faso (BF); Burma (BU); Burundi (BI); Cameroon (CM); Canada (CA); pe Verde (CV); Cayman Islands (KY); Central African Empire (CF); Chad (TD); ile (CL); Colombia (CO); Comoro Islands (KM); Congo (CG); Costa Rica (CR); ba (CU); Cyprus (CY); Czechoslovakia (CS); Democratic Kampuchea (KH); mocratic Peoples Rep. of Korea (KP); Democratic Yemen (YD); Denmark (DK); ibouti (DJ); Dominican Republic (DM); Ecuador (EC); Egypt (EG); El Salvador V); Equatorial Guinea (GQ); Estonia (EE); Ethiopia (ET); Falkland Islands K); Fiji (FJ); Finland (FI); France (FR); Gabon (GA); Gambia (GM); Georgia E); Germany (DE); Ghana (GH); Gibraltar (GI); Greece (GR); Grenada (GD); emala (GT); Guinea (GN); Guinea-Bissau (GW); Guyana (GY); Haiti (HT); uras (HN); Hong Kong (HK); Hungary (HU); Iceland (IS); India (IN); Indonesia D); Iran (IR); Iraq (IQ); Ireland (IE); Israel (IL); Italy (IT); Ivory Coast I); Jamaica (JM); Japan (JP); Jordan (JO); Kazakhstan (KZ); Kenya (KE); ribati (KI); Kuwait (KW); Kyrgyzstan (KG); Laos (LA); Latvia (LV); Lebanon

KW); Kyrgyzstan (KG); Laos (LA); Latvia (LV); Lebanon (LB); Lesotho (LS); iberia (LR); Libya (LY); Liechtenstein (LI); Lithuania (LT); Luxembourg (LU); adagascar (MG); Malawi (MW); Malaysia (MY); Maldives (MV); Mali (ML); Malta MT); Mauritania (MR); Mauritius (MU); Mexico (MX); Moldova, Republic of (MD); onaco (MC); Mongolia (MN); Montserrat (MS); Morocco (MA); Mozambique (MZ); amibia (NA); Nauru (NR); Nepal (NP); Netherlands (NL); Netherlands Antilles AN); New Zealand (NZ); Nicaragua (NI); Niger (NE); Nigeria (NG); Norway (NO); (OM); Pakistan (PK); Panama (PA); Papua New Guinea (PG); Paraguay (PY); le's Republic of China (CN); Peru (PE); Philippines (PH); Poland (PL); ortugal (PT); Qatar (QA); Republic of Korea (KR); Romania (RO); Russian ederation (RU); Rwanda (RW); Saint Lucia (LC); Samoa (WS); San Marino (SM); Sao ome and Principe (ST); Saudi Arabia (SA); Senegal (SN); Seychelles (SC); Sierra eone (SL); Sikkim (SK); Singapore (SG); Soloman Islands (SB); Somalia (SO); outh Africa (ZA); Spain (ES); Sri Lanka (LK); St. Kitt-Nevis (KN); St.Helena SH); St.Vincent and Grenadines (VC); Sudan (SD); Surinam (SR); Swaziland (SZ); weden (SE); Switzerland (CH); Syria (SY); Taiwan (TW); Tajikistan (TJ); anzania (TZ); Thailand (TH); Togo (TG); Tonga (TO); Trinidad and Tobago (TT); unisia (TN); Turkey (TR); Turkmenistan (TM); Turks & Caicos Islands (TC); valu (TV); U.S.S.R (SU); Uganda (UG); Ukraine (UA); United Arab Emirates (AE); ited Kingdom (GB); United States of America (US); Upper Volta (HV); Uruguay Y); Uzbekistan (UZ); Vanuatu (VU); Vatican City State (VA); Venezuela (VE); etnam (VN); Yemen Arab Republic (YE); Yugoslavia (YU); Zaire (ZR); Zambia M); Zimbabwe (ZW)

GAL-REP: Hansen, Bernd, Dr. Dipl.-Chem., Hoffmann, Eitle & Partner Patent- und chtsanwalte, Postfach 81 04 20, D-81904 Munchen Germany (DE) et al.

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FR A 2,446,110; Category A - Technological background
JP A 0,045,718; Category A - Technological background

SOURCE: Originates from the search report

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ABST:

ABST:

An antiviral agent containing at least one crude drug selected from the group consisting of (A1)the whole plant of Ainsliaea fragrans Champ., (A2)the rhizome of Alpinia officinarum Hance, (A3)the bark of Alyxia stellata Roem., (A4)the bark of Andrographis paniculate Nees, (A5)the root of Andropogon zizaniodes (L.) Urban, (A6)the rhizome of Anemarrhena asphodeloides Bunge, (A7)the leaf of Arctostaphylos uva-ursi (L.) Sprengel, (A8)the seed of Areca catechu L., (A9)the leaf of Artemisia princeps Pamp., (A10)the whole plant of Asarum heterotropoides F. Maekawa var. mandshuricum F. Maekawa, (A11)the rhizome of Belamcanda chinensis (L.) DC., (A12)the rhizome of Brainia insignis (Hook.) J. Sm., (A13)the seed of Brucea javanica (L.) Merr., (A14)the root of Bupleurum fakatum L., (A15)the bark of Caesalpinia sappan L., (A16)the leaf of Camellia japonica L., (A17)the bark of Cassia fistula L., (A18)the whole plant of Chamaesyce hyssopifolia, (A19)the bark and branch of Cinnamomum cassia Blume, (A20)the bark of Cinnamomum sintok Blume, (A21)the rhizome of Cnidium officinale Makino, (A22)the rhizome of Coptis chinensis Franch., (A23)the leaf of Cordia spinescens, (A24)the fruit of Cornus officinalis Sieb. et Zucc., (A25)the tuber of Corydalis hurtscharinorii Besser forma yanhusuo Y.H. Chou et C.C. Hsu, (A26)the fruit of Curculigo orchioides Gaertn., (A27)the rhizome of Curcuma aeroginosa Roxb., (A28)the rhizome of Curcuma xanthorrhiza Roxb., (A29)the rhizome of Cyrtomium fortunei J. Sm., (A30)the rhizome of Drynaria fortunei (Kunze) J. Smith, (A31)the rhizome of Dryopteris crassirhizoma Nakai, (A32)the fruit of Elaeocarpus grandiflorus Smith, (A33)the leaf of Elephantopus scaber L., (A34)the leaf of Epimedium koreanum Nakai, (A35)the leaf of Erythroxylum lucidum, (A35')the trunk of Erythroxylum citrifolium, (A36)the fruit of Evodia rutaecarpa Hook. f. et Thoms., (A37)the fruit of Foeniculum vulgare Mill., (A38)the fruit of Forsythia suspensa Vahl., (A39)the whole plant of Geranium thunbergii Sieb. et Zucc., (A40)the whole plant of Geum janonicum Thunb., (A41)the root and stolon of Glycyrrhiza uralensis Fisher (A42)the leaf of Hamelia axillaris Swartz, (A43)the branch and leaf of Jatropha curcas L., (A44)the bark of Juglans mandshurica Maxim., (A45)the root of Lithospermum erythrorhizon Sieb. et Zucc., (A46)the aerial part of Loranthus parasiticus (L.) Merr., (A47)the bark of Machilus thunbergii Sieb. et Zucc., (A48)the bark of Magnolia officinalis Rehd. et Wils., (A49)the rhizome of Matteuccia struthiopteris (L.) Todaro, (A50)the whole insect of Mylabris sidae Fabr., (A51)the root bark of Paeonia suffruticosa Andrews, (A52)the root of Panax ginseng C.A. Meyer, (A53)the bark of Parameria laevigata Moldenke, (A54)the leaf of Perilla frutescens Britton var. acuta Kudo, (A55)the bark of Phellodendron amurense Ruprecht, (A56)the aerial part of Physalis angulata L., (A57)the rhizome of Plagiogyria matsumureana Makino, (A58)the root of Platycodon grandiflorum (Jacquin) A. DC., (A59)the root of Polygala tenuifolia Willd., (A60)the root and rhizome of Polygonum cuspidatum Sieb. et Zucc., (A61)the

A60)the root and rhizome of Polygonum cuspidatum Sieb. et Zucc., (A61)the
oelen of Poria cocos Wolf, (A62)the spike of Prunella vulgaris L. subsp.
siatica Hara, (A63)the bark of Prunus jamasakura Siebold, (A64)the fruit of
runus mume Sieb. et Zucc., (A65)the root bark and fruit peel of Punica granatum
., (A66)the bark of Quercus acutissima Carruthers, (A67)the leaf of Quercus
alicina Blume, (A68)the fruit of Quisgualis indica L., (A69)the rhizome of
heum palmatum L., (A70)the gall of Rhus javanica L., (A71)the root of Salvia
iorrhiza Bunge, (A72)the leaf of Sarcandra glabra (Thunb.) Nakai, (A73)the
es of Schizonepeta tenuifolia Briquet, (A74)the root of Scutellaria
aicalensis Georgi, (A75)the whole plant of Serjania mexicana, (A76)the flower
ud of Sophora japonica L., (A77)the root of Sophora subprostrata Chun et T.
hen, (A78)the stem of Spatholobus suberectus Dunn, (A79)the rhizome of
truthiopteris niponica (Kunze) Nakai, (A80)the seed of Strychnos nux-vomica L.,
A81)the flower bud of Syzygium aromaticum (L.) Merr. et Perry, (A82)the bark of
erminalia arjuna Wight et Arn., (A83)the fruit peel of Terminalia belerica
oxb., (A84)the fruit of Terminalia chebula Retzus, (A85)the leaf and branch of
ncaria gambir Roxb., (A86)the whole plant of Usnea misaminensis Vain., (A87)the
ranch and leaf of Waltheria indica L., (A88)the flower and leaf of Woodfordia
loribunda Salisb., (A89)the rhizome of Woodwardia orientalis Sw., (A90)the
ruit peel of Zanthoxylum bungeanum Maxim., and (A91)the fruit of Zizyphus
ujuba Miller var. inermis Rehder.

UM:

ELD OF THE INVENTION

The present invention relates to an antiviral agent containing a crude
ug.

CKGROUND OF THE INVENTION

In recent years, opportunistic infectious diseases caused by viruses, such as
tomegalovirus (hereinafter abbreviated as CMV), in patients maintained on
munosuppressants, such as recipients of organ transplantation, have given rise
a problem. For instance, such infectious diseases have arisen the problem in
e first living liver transplantation conducted in Shimane Medical Collage,
pan.

t has been clinically observed that the incidence of CMV infectious diseases
es depending on the combination of immunosuppressants administered to
st-transplantation patients. Based on this observation, the present inventors
udied the influences of individual immunosuppressants and combinations
ereof on proliferation of CMV through in vitro testing systems. As a result,

thereof on proliferation of CMV through in vitro testing systems. As a result, of various known immunosuppressants, cyclosporine and predonine accelerated CMV proliferation, while mizoribine and azathioprine inhibited CMV proliferation. These results are in good agreement with case reports as described below. Therefore, it is believed that substances exhibiting antiviral activity in vitro also possess antiviral activity in vivo.

More specifically, the cases have been reported in which CMV infection developed in 100% of patients on cyclosporine + predonine therapy whereas the incidence of CMV infection was as low as 51.5% or 63.6% in patients on the therapy with cyclosporine + predonine in combination with mizoribine or azathioprine, respectively, suggesting the contribution of mizoribine or azathioprine to the anti-CMV effect (see Shiraki K., et al., rinsho to virus, Vol. 18, pp. 25-29, "men-ekifuzenjotai ni okeru virus no saikasseika (Re-activation of virus in immunodeficiency)" (1990); Shiraki K., et al., Biomedica., Vol. 5, pp. 65-69, "men-eki yokuseizai to CMV (Immunosuppressants and CMV)" (1990); Shiraki K., et al., Transplant. Proc., Vol. 22, pp. 1682-1685, "Effect of cyclosporine, azathioprine, mizoribine and predonine on replication of human cytomegalovirus" (1990); and Shiraki K., et al., Arch. Virol., Vol. 117, pp.165-171, "Immunosuppressive dose of azathioprine inhibits replication of human cytomegalovirus" (1991).

The like observation applies to FK 506 and cyclosporine as immunosuppressants. It has been reported that FK 506 has no or slight inhibitory influence on CMV growth (see Shiraki K., et al., J. Antibiotics, Vol. 44, pp. 909-911, "Effect of FK 506 replication of human cytomegalovirus in vitro" (1991)), while cyclosporine reveals the above-mentioned results.

According to the clinical report of Alessiani, et al., no difference was recognized in the incidence of bacterial and fungal infectious diseases between liver transplantation recipients on FK 506 + predonine therapy and those on cyclosporine + predonine therapy, while symptomatic CMV infectious disease developed in 0 out of 20 post-transplantation patients on the former therapy and 5 out of 20 post-transplantation patients on the latter therapy (see Alessiani M., et al., Transplant. Proc., Vol. 22, pp. 44-46, "Infection with FK 506 Immunosuppression; Preliminary results with primary therapy" (1990)). These clinical reports back up the CMV proliferation accelerating effect of cyclosporine observed in vitro.

Further, Bia, et al. made a case report that the incidence of CMV infection in post-transplantation patietns on azathioprine + steroid therapy was about half that in those on cyclosporine + steroid therapy, and no severe case was

half that in those on cyclosporine + steroid therapy, and no severe case was observed in the former, both groups of patients having received no anti-T cell globulin (see Bia M.J., et al., Transplantation, Vol. 40, pp. 610-614, "Effect of treatment with cyclosporine versus azathioprine on incidence and severity of cytomegalovirus infection post-transplantation" (1985)).

Development of CMV infection is largely influenced by the immune condition of the host, the degree of immune suppression, and the like and does not seem to be determined simply by the combination of immunosuppressants. Nevertheless, it is understood that the clinical observations of post-transplantation patients account for the influences of immunosuppressants on CMV proliferation in vitro more duly than expected.

The above situation implies possibility to alleviate CMV infectious disease by use of some of drugs currently employed as immunosuppressants which exhibit mild, while not potent, antiviral activity.

The reason why immunosuppressants essentially having weaker anti-CMV activity than general antiviral agents eventually exhibit effective anti-CMV activity will be explained below. Taking CMV-caused pneumonia for instance, it takes about 2 weeks for the very early stage (in which the X-ray picture of the chest demonstrates changes which retrospectively appear abnormal) to develop into the stage which is clinically recognized as CMV pneumonia. Tentatively setting the doubling time of CMV in the body at 72 hours (3 days), CMV increases 4 to 5 times within 2 weeks. Assuming that mizoribine or azathioprine controls CMV proliferation to controlled by administering mizoribine or azathioprine to one-thirty second through the 5-fold doubling period, i.e., about 3% of the amount of the virus in the case of using no mizoribine or azathioprine. It is considered natural that no CMV-caused disease occurs with such a small amount of CMV in patients on mizoribine or azathioprine therapy.

On the other hand, FK 506 hardly affects CMV proliferation, while cyclosporine accelerates CMV proliferation about twice. Accordingly, the CMV amount in the case of cyclosporine therapy will be 32 times that in the case of FK 506 therapy as calculated in the same manner as above. In addition, FK 506 is concentrated in the lung, the target organ of CMV infection, and the CMV proliferation is suppressed at that concentration by FK 506. This fact appears to contribute to the difference between FK 506 therapy and cyclosporine therapy.

Thus, it has now been revealed that use of an immunosuppressant having weak but effective anti-CMV activity possibly alleviates CMV infectious disease. Conventional studies have never been directed to the relationship between CMV

onventional studies have never been directed to the relationship between CMV
id immunosuppressants from this point of view.

On the other hand, traditional medicines (such as traditional Chinese
edicines and traditional Japanese medicines) (it is called crude drugs
ereinafter) have been used for therapy for a long number of years, and ample
nowledge of their usage, dosage, etc. has been accumulated. Many of crude
gs have minor or substantially no side effects. However, studies on antiviral
rity of crude drugs are rare. The literature on this subject now available
ncludes Ito M., et al., Antiviral Research, Vol. 7, pp. 127-137, "Inhibitory
ffect of glycyrrhizin on the in vitro infectivity and cytopathic activity of
ne human immunodeficiency virus HIV (HTLV-III/LAV)!", (1987); Takechi M. and
naka Y., Planta Medica, Vol. 42, pp. 69-74, "Purification and characterization
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thyl-3,4,5-trihydroxybenzoate, is a potent and highly specific inhibitor of
rpes simplex virus in vitro: I. Purification and characterization of methyl
llate from Sapium sebiferum", (1988); Nagai T., et al., Chem. Pharm. Bull.,
l. 38, pp. 1329-1332, "Inhibition of influenza virus sialidase and
nti-influenza virus activity by plant flavonoids", (1990); and Arai Y., et al.,
of Medical and Pharmaceutical Society for WAKAN-YAKU, Vol. 4, pp. 402-403,
ffect of Bezoar bovis against Chikungunya virus", (1987).

MMARY OF THE INVENTION

An object of the present invention is to provide an antiviral agent
ntaining a crude drug having effectiveness on viral diseases, taking the
ove-mentioned relationship between CMV and immunosuppressants as persuasive
d theoretical grounds for usefulness of some crude drugs which possess even
n-potent but mild antiviral activity in the treatment of virus-caused
seases.

Another object of the present invention is to provide an antiviral agent
ntaining a crude drug effective on a broad range of viruses.

The present inventors have conducted extensive investigations on a variety of
ude drugs, including traditional Chinese medicines, Japanese medicines, and
donesian medicines, which have conventionally been used as medicines, usage or
age of which is known, and which involve no or a little side effect. As a
lt, they have found among them crude drugs having antiviral activity and
us reached the present invention.

The present invention relates to an antiviral agent containing at least one

Zucc., (A61)the hoelen of Poria cocos Wolf, (A62)the spike of Prunella vulgaris L. subsp. asiatica Hara, (A63)the bark of Prunus jamasakura Siebold, (A64)the fruit of Prunus mume Sieb. et Zucc., (A65)the root bark and fruit peel of Punica granatum L., (A66)the bark of Quercus acutissima Carruthers, (A67)the leaf of Quercus salicina Blume, (A68)the fruit of Quisqualis indica L., (A69)the rhizome of Rheum palmatum L., (A70)the gall of Rhus javanica L., (A71)the root of Salvia miltiorrhiza Bunge, (A72)the leaf of Sarcandra glabra (Thunb.) Nakai, (A73)the [illegible]es of Schizonepeta tenuifolia Briquet, (A74)the root of Scutellaria [illegible]lensis Georgi, (A75)the whole plant of Serjania mexicana, (A76)the flower bud of Sophora japonica L., (A77)the root of Sophora subprostrata Chun et T. Chen, (A78)the stem of Spatholobus suberectus Dunn, (A79)the rhizome of Struthiopteris niponica (Kunze) Nakai, (A80)the seed of Strychnos nux-vomica L., (A81)the flower bud of Sysygium aromaticum (L.) Merr. et Perry, (A82)the bark of Terminalia arjuna Wight et Arn., (A83)the fruit peel of Terminalia belerica Roxb., (A84)the fruit of Terminalia chebula Retzus, (A85)the leaf and branch of Uncaria gambir Roxb., (A86)the whole plant of Usnea misaminensis Vain., (A87)the branch and leaf of Waltheria indica L., (A88)the flower and leaf of Woodfordia floribunda Salisb., (A89)the rhizome of Woodwardia orientalis Sw., (A90)the fruit peel of Zanthoxylum bungeanum Maxim., and (A91)the fruit of Zizyphus Jujuba Miller var. inermis Rehder (hereinafter referred to as group A).

The first embodiment of the present invention relates to an antiherpesviral agent containing at least one crude drug selected from the group consisting of (B1)the whole plant of Ainsliaea fragrans Champ., (B2)the rhizome of Alpinia officinarum Hance, (B3)the bark of Alyxia stellata Roem., (B4)the root of Andropogon zizaniodes (L.) Urban, (B5)the seed of Areca catechu L., (B6)the leaf of Artemisia princeps Pamp., (B7)the rhizome of Brainia insignis (Hook.) J. Sm., (B8)the seed of Brucea javanica (L.) Merr., (B9)the bark of Caesalpinia sappan L., (B10)the leaf of Camellia japonica L., (B11)the bark of Cassia fistula L., (B12)the whole plant of Chamaesyce hyssopifolia, (B13)the bark and branch of Cinnamomum cassia Blume, (B14)the bark of Cinnamomum sintok Blume, (B15)the rhizome of Coptis chinensis Franch., (B16)the leaf of Cordia spinescens, (B17)the rhizome of Cyrtomium fortunei J. Sm., (B18)the rhizome of Drynaria fortunei (Kunze) J. Smith, (B19)the rhizome of Dryopteris crassirhizoma Nakai, (B20)the fruit of Elaeocarpus grandiflorus Smith, (B21)the leaf of Epimedium koreanum Nakai, (B22)the leaf of Erythroxylum lucidum, (B22')the trunk of Erythroxylum citrifolium, (B23)the fruit of Foeniculum vulgare Mill., (B24)the whole plant of Geranium thunbergii Sieb. et Zucc., (B25)the whole plant of Geum [illegible]aicum Thunb., (B26)the leaf of Hamelia axillaris Swartz, (B27)the branch and leaf of Jatropha curcas L., (B28)the bark of Juglans mandshurica Maxim., (B29)the bark of Machilus thunbergii Sieb. et Zucc., (B30)the root bark of Paeonia suffruticosa Andrews, (B31)the leaf of Perilla frutescens Britton var.

Paeonia suffruticosa Andrews, (B31)the leaf of Perilla frutescens Britton var. acuta Kudo, (B32)the bark of Phellodendron amurense Ruprecht, (B33)the rhizome of Plagiogyria matsumureana Makino, (B34)the root of Polygala tenuifolia Willd., (B35)the root and rhizome of Polygonum cuspidatum Sieb. et Zucc., (B36)the spike of Prunella vulgaris L. subsp. asiatica Hara, (B37)the root bark and fruit peel of Punica granatum L., (B38)the bark of Quercus acutissima Carruthers, (B39)the rhizome of Rheum palmatum L., (B40)the gall of Rhus javanica L., (B41)the root of Salvia miltiorrhiza Bunge, (B42)the leaf of Sarcandra glabra (Thunb.) Nakai, ( )the flores of Schizonepeta tenuifolia Briquet, (B44)the whole plant of Serjania mexicana, (B45)the stem of Spatholobus suberectus Dunn, (B46)the bark of Terminalia arjuna Wight et Arn., (B47)the fruit peel of Terminalia belerica Roxb., (B48)the fruit of Terminalia chebula Retzus, (B49)the branch and leaf of Waltheria indica L., (B50)the flower and leaf of Woodfordia floribunda Salisb., (B51)the rhizome of Woodwardia orientalis Sw., and (B52)the fruit peel of Zanthoxylum bungeanum Maxim. (hereinafter referred to as group B).

The second embodiment of the present invention relates to an antipolioviral agent containing at least one crude drug selected from the group consisting of (C1)the rhizome of Alpinia officinarum Hance, (C2)the bark of Andrographis paniculate Nees, (C3)the root of Andropogon zizaniodes (L.) Urban, (C4)the rhizome of Anemarrhena asphodeloides Bunge, (C5)the leaf of Arctostaphylos uva-ursi (L.) Sprengel, (C6)the seed of Areca catechu L., (C7)the leaf of Artemisia princeps Pamp., (C8)the whole plant of Asiasarum heterotropoides F. Maekawa var. mandshuricum F. Maekawa, (C9)the rhizome of Belamcanda chinensis (L.) DC., (C10)the rhizome of Brainia insignis (Hook.) J. Sm., (C11)the seed of Brucea javanica (L.) Merr, (C12)the bark of Caesalpinia sappan L., (C13)the bark of Cassia fistula L., (C14)the bark of Cinnamomum sint++1999293106801ok Blume, (C15)the rhizome of Coptis chinensis Franch., (C16)the fruit of Cornus officinalis Sieb. et Zucc., (C17)the tuber of Corydalis hurtscharinorii Besser forma yanhusuo Y.H. Chou et C.C. Hsu, (C18)the fruit of Curculigo orchioides Gaertn., (C19)the rhizome of Curcuma aeroginosa Roxb., (C20)the rhizome of Curcuma xanthorrhiza Roxb., (C21)the rhizome of Cyrtomium fortunei J. Sm., (C22)the rhizome of Dryopteris crassirhizoma Nakai, (C23)the fruit of Elaeocarpus grandiflorus Smith, (C24)the leaf of Elephantopus scaber L., (C25)the fruit of Evodia rutaecarpa Hook. f. et Thoms., (C26)the fruit of Foeniculum vulgare Mill., (C27)the fruit of Forsythia suspensa Vahl., (C28)the whole plant of Geranium thunbergii Sieb. et Zucc., (C29)the whole plant of Geum japonicum Thunb., (C30)the bark of Juglans mandshurica Maxim., (C31)the root of Lithospermum erythrorhizon Sieb. et Zucc., (C32)the aerial part of Loranthus parasiticus (L.) Merr., (C33)the bark of Machilus thunbergii Sieb. et Zucc., (C34)the rhizome of Matteuccia struthiopteris (L.) Todaro, (C35)the whole insect of Mylabris sidae Fabr., (C36)the root bark of Paeonia suffruticosa

insect of Mylabris sidae Fabr., (C36)the root bark of Paeonia suffruticosa Andrews, (C37)the bark of Parameria laevigata Moldenke, (C38)the bark of Phellodendron amurense Ruprecht, (C39)the aerial part of Physalis angulata L., (C40)the rhizome of Plagiogyria matsumureana Makino, (C41)the root of Polygala tenuifolia Willd., (C42)the root and rhizome of Polygonum cuspidatum Sieb. et Zucc., (C43)the bark of Prunus jamasakura Siebold, (C44)the fruit of Prunus mume Sieb. et Zucc., (C45)the root bark and fruit peel of Punica granatum L., (C46)the bark of Quercus acutissima Carruthers, (C47)the leaf of Quercus [illegible]cina Blume, (C48)the fruit of Quisqualis indica L., (C49)the rhizome of Rheum palmatum L., (C50)the gall of Rhus javanica L., (C51)the root of Scutellaria baicalensis Georgi, (C52)the flower bud of Sophora japonica L., (C53)the root of Sophora subprostrata Chun et T. Chen, (C54)the stem of Spatholobus suberectus Dunn, (C55)the rhizome of Struthiopteris niponica (Kunze) Nakai, (C56)the seed of Strychnos nux-vomica L., (C57)the flower bud of Syzygium aromaticum (L.) Merr. et Perry, (C58)the bark of Terminalia arjuna Wight et Arn., (C59)the fruit peel of Terminalia belerica Roxb., (C60)the fruit of Terminalia chebula Retzus, (C61)the leaf and branch of Uncaria gambir Roxb., (C62)the whole plant of Usnea misaminensis Vain., (C63)the flower and leaf of Woodfordia floribunda Salisb., (64)the rhizome of Woodwardia orientalis Sw., and (C65)the fruit peel of Zanthoxylum bungeanum Maxim. (hereinafter referred to as group C).

The third embodiment of the present invention relates to an anti-measles virus agent containing at least one crude drug selected from the group consisting of (D1)the seed of Areca catechu L., (D2)the leaf of Artemisia princeps Pamp., (D3)the rhizome of Belamcanda chinensis (L.) DC., (D4)the rhizome of Brainia insignis (Hook.) J. Sm., (D5)the seed of Brucea javanica (L.) Merr, (D6)the bark of Caesalpinia sappan L., (D7)the bark of Cassia fistula L., (D8)the bark of Cinnamomum sintok Blume, (D9)the rhizome of Cnidium officinale Makino, (D10)the rhizome of Coptis chinensis Franch., (D11)the rhizome of Cyrtomium fortunei J. Sm., (D12)the fruit of Elaeocarpus grandiflorus Smith, (D13)the leaf of Elephantopus scaber L., (D14)the fruit of Foeniculum vulgare Mill., (D15)the fruit of Forsythia suspensa Vahl., (D16)the whole plant of Geranium thunbergii Sieb. et Zucc., (D17)the root and stolon of Glycyrrhiza uralensis Fisher, (D18)the bark of Juglans mandshurica Maxim., (D19)the bark of Magnolia officinalis Rehd. et Wils., (D20)the whole insect of Mylabris sidae Fabr., (D21)the root bark of Paeonia suffruticosa Andrews, (D22)the bark of Phellodendron amurense Ruprecht, (D23)the rhizome of Plagiogyria matsumureana Makino, (D24)the root of Platycodon grandiflorum (Jacquin) A. DC., (D25)the root of Polygala tenuifolia Willd., (D26)the root bark and fruit peel of Punica granatum L., (D27)the bark of Quercus acutissima Carruthers, (D28)the rhizome of Rheum palmatum L., (D29)the gall of Rhus javanica L., (D30)the root of

of Rheum palmatum L., (D29)the gall of Rhus javanica L., (D30)the root of Scutellaria baicalensis Georgi, (D31)the stem of Spatholobus suberectus Dunn, (D32)the flower bud of Syzygium aromaticum (L.) Merr. et Perry, (D33)the bark of Terminalia arjuna Wight et Arn., (D34)the fruit peel of Terminalia belerica Roxb., (D35)the fruit of Terminalia chebula Retzus, (D36)the flower and leaf of Woodfordia floribinda Salisb., (D37)the rhizome of Woodwardia orientalis Sw., and (D38)the fruit of Zizyphus jujuba Miller var. inermis Rehder (hereinafter referred to as group D).

The forth embodiment of the present invention relates to an anti-varicella-zoster virus agent containing at least one crude drug selected from the group consisting of (E1)the rhizome of Alpinia officinarum Hance, (E2)the seed of Areca catechu catechu L., (E3)the leaf of Artemisia princeps Pamp., (E4)the root of Bupleurum fakatum L., (E5)the bark of Cassia fistula L., (E6)the rhizome of Coptis chinensis Franch., (E7)the rhizome of Cyrtomium fortunei J. Sm., (E8)the rhizome of Drynaria fortunei (Kunze) J. Smith, (E9)the whole plant of Geranium thunbergii Sieb. et Zucc., (E10)the root and stolon of Glycyrrhiza uralensis Fisher, (E11)the bark of Juglans mandshurica Maxim., (E12)the root bark of Paeonia suffruticosa Andrews, (E13)the root of Panax ginseng C.A. Meyer, (E14)the bark of Phellodendron amurense Ruprecht, (E15)the rhizome of Plagiogyria matsumureana Makino, (E16)the root of Polygala tenuifolia Willd., (E17)the root and rhizome of Polygonum cuspidatum Sieb. et Zucc., (E18)the hoelen of Poria cocos Wolf, (E19)the root bark and fruit peel of Punica granatum L., (E20)the bark of Quercus acutissim Carruthers, (E21)the rhizome of Rheum palmatum L., (E22)the gall of Rhus javanica L., (E23)the flower bud of Syzygium aromaticum (L.) Merr. et Perry, (E24)the bark of Terminalia arjuna Wight et Arn., (E25)the fruit of Terminalia chebula Retzus, (E26)the rhizome of Woodwardia orientalis Sw., and (E27)the rhizome of Dryopteris crassirhizoma Nakai (hereinafter referred to as group E).

The fifth embodiment of the present invention relates to an anti-CMV agent containing at least one crude drug selected from the group consisting of (F1)the seed of Areca catechu L., (F2)the leaf of Artemisia princeps Pamp., (F3)the bark of Cassia fistula L., (F4)the rhizome of Coptis chinensis Franch., (F5)the rhizome of Cyrtomium fortunei J. Sm., (F6)the rhizome of Drynaria fortunei (Kunze) J. Smith, (F7)the whole plant of Geranium thunbergii Sieb. et Zucc., (F8)the whole plant of Geum japonicum Thunb., (F9)the bark of Juglans mandshurica Maxim., (F10)the bark of Machilus thunbergii Sieb. et Zucc., (F11)the root bark of Paeonia suffruticosa Andrews, (F12)the bark of Phellodendron amurense Ruprecht, (F13)the rhizome of Plagiogyria matsumureana Makino, (F14)the root of Polygala tenuifolia Willd., (F15)the root and rhizome of Polygonum cuspidatum Sieb. et Zucc., (F16)the root bark and fruit peel of

of Polygonum cuspidatum Sieb. et Zucc., (F16)the root bark and fruit peel of Punica granatum L., (F17)the bark of Quercus acutissima Carruthers, (F18)the rhizome of Rheum palmatum L., (F19)the gall of Rhus javanica L., (F20)the flower bud of Syzygium aromaticum (L.) Merr. et Perry, (F21)the bark of Terminalia arjuna Wight et Arn., (F22)the fruit of Terminalia chebula Retzus, and (F23)the rhizome of Woodwardia orientalis Sw. (hereinafter referred to as group F).

The sixth embodiment of the present invention relates to an anti-DNA virus and anti-RNA virus agent containing at least one crude drug selected from the group consisting of (G1)the seed of Areca catechu L., (G2)the leaf of Artemisia princeps Pamp., (G3)the rhizome of Brainia insignis (Hook.) J. Sm., (G4)the seed of Brucea javanica (L.) Merr., (G5)the bark of Caesalpinia sappan L., (G6)the bark of Cassia fistula L., (G7)the bark of Cinnamomum sintok Blume, (G8)the rhizome of Coptis chinensis Franch., (G9)the rhizome of Cyrtomium fortunei J. Sm., (G10)the fruit of Elaeocarpus grandiflorus Smith, (G11)the fruit of Foeniculum vulgare Mill., (G12)the whole plant of Geranium thunbergii Sieb. et Zucc., (G13)the bark of Juglans mandshurica Maxim., (G14)the root bark of Paeonia suffruticosa Andrews, (G15)the bark of Phellodendron amurense Ruprecht, (G16)the rhizome of Plagiogyria matsumureana Makino, (G17)the root of Polygala tenuifolia Willd., (G18)the root bark and fruit peel of Punica granatum L., (G19)the bark of Quercus acutissima Carruthers, (G20)the rhizome of Rheum palmatum L., (G21)the gall of Rhus javanica L., (G22)the stem of Spatholobus suberectus Dunn, (G23)the flower bud of Syzygium aromaticum (L.) Merr. et Perry, (G24)the bark of Terminalia arjuna Wight et Arn., (G25)the fruit peel of Terminalia belerica Roxb., (G26)the fruit of Terminalia chebula Retzus, (G27)the flower and leaf of Woodfordia floribunda Salisb., and (G28)the rhizome of Woodwardia orientalis Sw. (hereinafter referred to as group G).

The seventh embodiment of the present invetion relates to an antiherpesviral agent containing at least one crude drug selected from the group B as mentioned above and the other antiviral agent.

DETDESC:
DETAILED DESCRIPTION OF THE INVENTION

All viruses are classified into DNA viruses and RNA viruses. Families included under DNA viruses include Poxviridae, Herpesviridae, Adenoviridae, Papovaviridae, Hepadnaviridae, and Parvoviridae. Families included under RNA viruses include Reoviridae, Picornaviridae, Togaviridae, Flaviviridae, Coronaviridae, Rhabdoviridae, Paramyxoviridae, Orthomyxoviridae, Filoviridae, Bunyaviridae, Arenaviridae, Caliciviridae and Retroviridae.

The present invention relates to an antiviral agent containing at least one crude drug selected from the group consisting of (A1)the whole plant of Ainsliaea fragrans Champ., (A2)the rhizome of Alpinia officinarum Hance, (A3)the bark of Alyxia stellata Roem., (A4)the bark of Andrographis paniculate Nees, (A5)the root of Andropogon zizaniodes (L.) Urban, (A6)the rhizome of Anemarrhena asphodeloides Bunge, (A7)the leaf of Arctostaphylos uva-ursi (L.) Sprengel, (A8)the seed of Areca catechu L., (A9)the leaf of Artemisia princeps Pamp., (A10)the whole plant of Asiasarum heterotropoides F. Maekawa var. mandshuricum F. Maekawa, (A11)the rhizome of Belamcanda chinensis (L.) DC., (A12)the rhizome of Brainia insignis (Hook.) J. Sm., (A13)the seed of Brucea javanica (L.) Merr., (A14)the root of Bupleurum fakatum L., (A15)the bark of Caesalpinia sappan L., (A16)the leaf of Camellia japonica L., (A17)the bark of Cassia fistula L., (A18)the whole plant of Chamaesyce hyssopifolia, (A19)the bark and branch of Cinnamomum cassia Blume, (A20)the bark of Cinnamomum sintok Blume, (A21)the rhizome of Cnidium officinale Makino, (A22)the rhizome of Coptis chinensis Franch., (A23)the leaf of Cordia spinescens, (A24)the fruit of Cornus officinalis Sieb. et Zucc., (A25)the tuber of Corydalis hurtscharinorii Bessor forma yanhusuo Y.H. Chou et c.c. Hsu, (A26)the fruit of Curculigo orchioides Gaertn., (A27)the rhizome of Curcuma aeroginosa Roxb., (A28)the rhizome of Curcuma xanthorrhiza Roxb., (A29)the rhizome of Cyrtomium fortunei J. Sm., (A30)the rhizome of Drynaria fortunei (Kunze) J. Smith, (A31)the rhizome of Dryopteris crassirhizoma Nakai, (A32)the fruit of Elaeocarpus grandiflorous Smith, (A33)the leaf of Elephantopus scaber L., (A34)the leaf of Epimedium koreanum Nakai, (A35)the leaf of Erythroxylum lucidum, (A35')the trunk of Erythroxylum citrifolium, (A36)the fruit of Evodia rutaecarpa Hook. f. et Thoms., (A37)the fruit of Foeniculum vulgare Mill., (A38)the fruit of Forsythia suspensa Vahl., (A39)the whole plant of Geranium thunbergii Sieb. et Zucc., (A40)the whole plant of Geum japonicum Thunb., (A41)the root and stolon of Glycyrrhiza uralensis Fisher (A42)the leaf of Hamelia axillaris Swartz, (A43)the branch and leaf of Jatropha curcas L., (A44)the bark of Juglans mandshurica Maxim., (A45)the root of Lithospermum erythrorhizon Sieb. et Zucc., (A46)the aerial part of Loranthus parasiticus (L.) Merr., (A47)the bark of Machilus thunbergii Sieb. et Zucc., (A48)the bark of Magnolia officinalis Rehd. et Wils., (A49)the rhizome of Matteuccia struthiopteris (L.) Todaro, (A50)the whole insect of Mylabris sidae Fabr., (A51)the root bark of Paeonia suffruticosa Andrews, (A52)the root of Panax ginseng C.A. Meyer, (A53)the bark of Parameria laevigata Moldenke, (A54)the leaf of Perilia frutescens Britton var. acuta Kudo, (A55)the leaf of Phellodendron amurense Ruprecht, (A56)the aerial part of Physalis alata L., (A57)the rhizome of Plagiogyria matsumureana Makino, (A58)the root of Platycodon grandiflorum (Jacquin) A. DC., (A59)the root of Polygala tenuifolia Willd., (A60)the root and rhizome of Polygonum cuspidatum Sieb. et Zucc., (A61)the hoelen of Poria cocos Wolf, (A62)the spike of Prunella vulgaris

Families belonging to DNA viruses have many basic traits in common in DNA
:plication mechanism. RNA viruses also have characteristics in common with each
:her in the growth step. The common characteristics include a number of
:ll-dependent DNA replication-related enzymes, complementarily acting cell
ıctors, and RNA synthesis by RNA dependent RNA polymerase (RNA replicase), and
› on. Therefore, a drug exhibiting inhibitory activity on proliferation of a
ypical DNA virus, e.g., the Herepesviridae, or a typical RNA virus, e.g., the
rnaviridae, is expected to be effective as an antiviral agent common to a
range of DNA or RNA viruses.

Viruses belonging to Picornaviridae include poliovirus, echovirus, coxsackie
.rus, enterovirus, and rhinovirus. Diseases caused by these viruses include
:ute anterior poliomyelitis, hand-foot-and-mouth disease, herpangina,
opericarditis, epidermic myalgia, enanthema, acute hemorrhagic conjuctivitis,
ummer cold, aseptic meningitis, hepatitis type A, coryza, respirator infectious
seases, and the like.

Viruses-zoster belonging to Paramyxoviridae include parainfluenze virus,
mps virus, measles virus, and RS virus. Diseases caused by theme viruses
clude pharyngitis, upper respiratory tract infectious disease, bronchitis,
eumonia, measles, mumps, and acute respiratory tract infectious disease.

Varicella virus belongs to the family Herpesviridae and causes varicella in
ildren aged between 2 and 8 easily by droplet infection or contagen. Herpes
ster mostly occurs in adults having once suffered from varicella and aged over
.

CMV also belongs to the family Herpesviridae. Besides the above-mentioned
oblem, if a woman is first infected with CMV during pregnancy, it sometiems
ppens that the fetus is directly infected to develop congenital cytomegalic
clusion body disease, etc. Further, CMV often plays a main role in
portunistic infection in acquired immunodeficiency syndrome (AIDS).

The crude drugs belonging to group B have antiherpesviral activity; those of
oup C antipolioviral activity; those of group D anti-measles virus activity;
ose of group E anti-varicella-zoster virus activity; those of group F anti-CMV
tivity; and those of group G anti-DNA virus and anti-RNA virus activity. Group
cludes all crude drugs of the present invention.

The dosage, administration route, etc. of the crude drugs included under
oup A, inclusive of groups B to G, are well known, and the known dose levels
oduce no or slight side effects.

roduce no or slight side effects.

2 to 10g of the crude drug according to the present invention is added to ater at a ratio of 50 ml water per g of the crude drug, and the mixture is oiled and concentrated to half the original volume. The resulting extract can e orally administered three times a day at a dose of one-third of the extract.

n particular, as hereinafter described, when at least one of the crude drugs roup B is used in combination of acyclovir, a known antiviral agent, there s produced synergistic antiviral activity, by which the requisite dose of cyclovir can be reduced, and appearance of acyclovir-resistant viruses will be nhibited.

Accordingly, the crude drug according to the present invention whose fraction aving been absorbed through the digestive tracts exhibits antiviral activity, r a combination of such a crude drug and other known antiviral agent is onsidered effective in prevention and treatment of the above-mentioned diseases aused by herpes simplex virus (e.g., herpes simplex and its complication), iseases caused by varicella-zoster virus (e.g., varicella, shingles and mplications thereof), and diseases caused by CMV (e.g., pneumonia, hepatitis, id conjunctivitis).

Test Examples of the present invention are described below. All the plants id insects used in the tests were purchased at the market. The part of the ants or insects used was as described above.
eparation of Extract:

Each plant or insect was extracted at reflux with distilled water under a utral condition, followed by concentration and drying to obtain an aqueous tract of the crude drug. It is generally spread to use methanol in place of ter as an extracting solvent for obtaining hydrophobic substances in higher ncentrations. The test of antiviral activity was also conducted on the thanol extract of some of crude drugs.

For example, 100 g of the rhizome of Cyrtomium fortunei J. Sm.<#> (available the Hong Kong market) was extracted at reflux with 1.5 P of distilled water, d the extract was concentrated at 40C under reduced pressure and lyophilized btain 12.3 g of an aqueous extract of Cyrtomium fortunei J. Sm.<#> (the term act" hereinafter used means an aqueous extract unless otherwise specified). rtomium fortunei J. Sm. with a sharp mark means a mixture of Brainia insignis ook.) J. Sm., Cyrtonium fortunei J. Sm., Dryopteris crassirhizoma Nakai, tteuccia struthiopteris (L.) Todaro, Plagiogyria matsumureana Makino,

Matteuccia struthiopteris (L.) Todaro, Plagiogyria matsumureana Makino, Struthiopteris niponica (Kunze) Nakai and Woodwardia orientalis Sw. The Cyrtomium fortunei J. Sm.<#> is available on the Hong Kong market.

A methanol extract was obtained by, for example, extracting 20 g of a crude drug with 500 mP of methanol for 3 hours and removing methanol by distillation under reduced pressure.

Each crude drug extract was ground and suspended in water in a prescribed concentration. The suspension was heated in a boiling water bath for 10 minutes and centrifuged (3000 rpm x 10 min.). The resulting supernatant, an extract solution, was used for the antiviral test.

Test Virus:

Herpes simplex virus type I (Seibert strain), poliovirus (vaccine strain and Sabin strain), measles virus (vaccine strain and Tanabe strain), Varicella-zoster virus (Kawaguchi strain), and CMV (Town strain) were used.

Vero cells or HEL cells derived from human embryonal lungs were infected with each virus except varicella-zoster virus. After incubation for a prescribed time the infected cells were subjected to freezing and thawing three times, followed by centrifugation (3000 rpm x 15 mins.). The supernatant was used as a virus suspension.

A varicella-zoster virus suspension was prepared according to the method of Shiraki K and Takahashi M., J. Gen. Virol., Vol. 61, pp. 271-275, "Virus particles and glycoproteines excreted from cultured cells infected with varicella-zoster virus".

The thus prepared virus liquids were preserved, if necessary, at -80C.

Cells to be Infected with Virus:

1. Vero cells originated from the kidney of African green monkey were cultured in a minimum essential medium (hereinafter abbreviated as MEM) containing 5% bovine fetal serum (BFS).

2. Vero E6 cells were obtained by cloning of the above-mentioned Vero cells have high susceptibility to infection with various viruses.

3. HEL cells originated from human embryonal lungs which have high susceptibility to infection with varicella-zoster virus and CMV.

Cultivation of all these cells was conducted in COincubator at 37C.

Plaque Formation Test Method:

0.2 mP of a virus suspension++1999293106801diluted to 100 PFU/0.2 mP was inoculated to a monolayer culture of Vero cells, Vero E6 cells or HEL cells in a plastic dish having a diameter of 60 mm. The virus was allowed to adsorb on the monolayer cells at 37C for 1 hour.

After adsorption, 5 mP of 2% BFS-added MEM containing each extract solution in a prescribed concentration and 0.8% methyl cellulose was pliled on the monolayer cells, and the system was cultured at 37C for 2 to 5 days to observe plaque formation. The cultured monolayer cells were fixed with formalin and stained with a 0.03% Methylene Blue solution for measuring the number of plaques.

Where the above-mentioned test was replicated several times with the same concentration of the extract solution, the results obtained sometimes show slight scatter. This seems attributed to the error in detection and judgement of plaques due to slight variation of susceptibility depending on the cultivation conditions, such as the lot of serum, the culture plate, the cell density, etc.

TEST EXAMPLE 1

Antiherpesviral activity of the crude drugs shown in Table 1 at a concentration shown were examined by using Vero cells or Vero E6 cells by the plaque formation test. The results obtained are shown in Table 1 below. It can be seen from the Table that the extract of the crude drug according to the present invention significantly reduced the efficiency of plaque formation at such a concentration as low as 500 Ng/mP or less and thus exhibited herpesvirus growth inhibitory activity.

TEST EXAMPLE 2

Antipolioviral activity of the crude drugs shown in Table 2 (group C) was determined in the same manner as in Test Example 1. The results are shown in the Table.

The crude drug extract solution shown in Table 2 showed cytotoxicity at a concentration of about 300 Ng/mP or 500g/mP. However, from the fact that these crude drugs have been orally administered for years with no or only slight side effects observed, cytotoxicity of these crude drugs appears to give rise to no problem.

Through screening various crude drugs for antiviral activity against

Through screening various crude drugs for antiviral activity against poliovirus as a typical species of the viruses belonging to the family Picornaviridae as described above, medicines having antiviral activity against viruses belonging to Picornaviridae and having the similar physicochemical properties and proliferation mechanism in common can be developed.

The crude drugs belonging to group C whose fraction absorbed through the digestive tract exhibits antiviral activity against poliovirus are considered effective for prevention and treatment of the diseases caused by viruses belonging to the family Picornaviridae.

TEST EXAMPLE 3

Anti-measles virus activity of the crude drugs shown in Table 3 (group D) was determined in the same manner as in Test Example 1. The results obtained are shown in Table 3. The crude drugs belonging to group D were thus proved to have anti-measles virus activity.

Through screening various crude drugs for antiviral activity against measle virus as a typical species of the viruses belonging to the family Paramyxoviridae as described above, medicines having antiviral activity against viruses belonging to Paramyxoviridae and having the similar physicochemical properties and proliferation mechanism in common can be developed.

The crude drugs belonging to group D whose fraction absorbed through the digestive tract exhibits antiviral activity against measle virus are considered effective for prevention and treatment of the diseases caused by viruses belonging to the family Paramyxoviridae.

TEST EXAMPLE 4

Anti-varicella-zoster virus activity of the crude drugs shows in Table 4 (group E) was determined in the same manner as in Test Example 1. The results obtained are shown in Table 4. The crude belonging to group E were thus proved to have anti-varicella-zoster virus activity. In the case of using Plagiogyria matsumureana Makino, methanol extract was used in place of water-extract.

TEST EXAMPLE 5

Anti-CMV activity of the crude drugs shown in Table 5 (group F) was determined in the same manner as in Test Example 1. The results obtained are shown in Table 5. From these results, the crude drugs belonging to group F were proved to have anti-CMV activity. In the case of using Plagiogyria matsumureana Makino, methanol extract was used in place of water extract.

TEST EXAMPLE 6

EST EXAMPLE 6

Antiviral activity of the crude drugs shown in Table 6 below was examined in he same manner as in Test Example 1, except for using Vero E6 cells and hanging the concentration of the extract solution. The results obtained are hown in Table 6. It can be seen that the extract of the crude drug according to he present invention significantly reduced the efficiency of plaque formation much a concentration as low as 300 Ng/mP or less and thus exhibited antiviral vity.

Cytotoxicity of each extract solution was also determined. While some of the xtract solutions tested showed cytotoxicity at a concentration of 300 Ng/mP, hey exhibited antiviral activity at low concentrations showing no cytotoxicity.

EST EXAMPLE 7

Antiherpesviral activity (anti-herpesvirus type I activity, nti-varicella-zoster virus activity, and anti-CMV activity) of the crude drugs hown in Table 7 below were examined by the plaque formation test. The nti-herpesvirus type I activity test was carried out using Vero E6 cells and an xtract solution diluted to 300 Ng/mP except where noted; and the nti-varicella-zoster virus activity test and anti-CMV activity test were arried out by using HEL cells and an extract solution diluted to 200 Ng/mP. The esults obtained are shown in Table 7. As can be seen from the Table, the xtract of the crude drug according to the present invention significantly educed the efficiency of plaque formation at such a concentration as low as 500 g/mP or less and thus exhibited antiherpesviral activity. Further, Cyrtomium ortunei J. Sm. exhibited similar effects in its methanol extract and aqueous xtract, suggesting that a crude drug whose methanol extract exhibits antiviral ctivity also exhibits antiviral activity in its aqueous extract as well.

EST EXAMPLE 8

Antiherpesviral activity of a combination of an extract solution of Cyrtomium ortunei (Kunze) J. Smith<#> (hereinafter abbreviated as Cryt. f.<#>) and an xtract solution of the crude drug shown in Table 8 was determined using erpesvirus type I and Vero E6 cells. Each extract solution was diluted to 100 g/mP in a single use, and in a combined use, two extract solutions each having concentration of 100 Ng/mPwere mixed. The results obtained are shown in Table

Antiherpesviral activity of a combination of Cryt. f.<#> and Artemisia inceps Pamp. was also determined in the same manner, except for using HEL lls, varicella-zoster virus, and CMV. The results obtained are shown in Table

cells, varicella-zoster virus, and CMV. The results obtained are shown in Table 9.

As is apparent from Tables 8 and 9, a combination of Cryt. f.<#> and other crude drugs showed appreciable synergism.

TEST EXAMPLE 9

Because the crude drugs according to the present invention are usually administered orally, the components which are absorbed through the gastrointestinal tract appear to be effective. Accordingly, the antiherpesviral activity of the serum prepared from the blood of a guinea pig having orally or intragastrointestinally received an extract solution of Cryt. f.<#> was determined according to the following animal test method.

For comparison, the antiherpesviral activity of the serum prepared in the same manner, except for replacing the Cryt. f.<#> extract with acyclovir ("Zovirax 200" sold by Wellcome Co.) (hereinafter abbreviated as Acv) known as an antiviral agent, was also determined. Acv tablets were ground to powder in a mortar and suspended in water or a crude drug extract solution in a prescribed concentration.

Animal Test Method:

Laparotomy was performed on a Hartley female guinea pig (body weight: 300-350 g) under etherization. Into each of the stomach, the small intestine, and the large intestine was infused 10 mP of water, an Acv suspension, a Cryt. f.<#> extract solution, or a mixed solution of Acv and the extract solution, and the abdomen was immediately closed. Two hours from the operation, blood was taken from the heart. The serum separated from the blood was inactivated by heating at 56C for 30 minutes.

Vero cells was monolayer-cultured in a 25 cm-volume plastic flask and infected with 0.01 PFU/mP of herpesvirus. After adsorption for 1 hour, the infected cells were cultured in MEM containing 30 to 40% of the above-prepared inactivated serum. After cultivation for a given time (1 to 4 days), the cells were destroyed by freezing and thawing three times, followed by centrifugation. The amount of the virus in the supernatant liquor was measured by the plaque formation test using Vero cells. The results obtained are shown in Tables 10 and 11.

As is apparent from the results in Tables 10 and 11, antiherpesviral activity was displayed in both the Cryt. f.<#> group and the Acv group, and the combined use of Cryt. f.<#> and Acv showed significant synergism.

ombined use of Cryt. f.<#> and Acv showed significant synergism.

EST EXAMPLE 10

Since the antiherpesviral activity of Acv is known, whether or not the ynergism shown in Test Example 9 had been attributed to the improvement of Acv bsorption by the extract solution of Cryt. f.<#> was examined by measuring Acv oncentration in blood when Acv was administered alone or in combination with a f.<#> extract solution.

An Acv suspension or a mixture of Acv and an extract solution of Cryt. f.<#> as orally or intragastrointestinally administered to a guinea pig. One hour rom the administration, blood was taken from the heart, and an inactivated erum was prepared therefrom in the same manner as in Test Example 9.

To a 100 Nl aliquot of the serum were added 100 NP of acetonitrile and 20 NP f a 100 mM sodium acetate solution, and the mixture was centrifuged (10000 rpm 10 mins.). The supernatant liquor (150 NP) was evaporated to dryness in an vaporator, and the residue was dissolved in 25 NP of a solvent for reverse hase liquid chromatography which contained 16.7 mM of adenine as an internal tandard to prepare a sample solution.

The sample solution was separated into Acv and adenine by reverse phase iquid chromatography in a usual manner. Each peak area was measured with an utomatic integrator, and the Acv content was obtained from the previously repared calibration curve. The results obtained are shown in Table 12. From the esults in Table 12, it is not recognized that the Acv absorption was ccelerated by the combined use of the Cryt. f.<#> extract solution. It is herefore considered that a Cryt. f.<#> extract solution per se possesses ntiherpesviral activity in its absorbable fraction.

Further, 0.5 mP of an Acv preparation or 0.5 mP of a mixture of Acv and a aried concentration of Cryt. f.<#> extract solution was orally administered to 6-weed-old DDY male mouse, and the Acv concentration in blood was measured in ne same manner as described above. The results obtained are shown in Table 13. s is apparent from Table 13, no increase in Acv concentration in blood was bserved in the group having received Acv combined with Cryt. f.<#>. ccordingly, the Cryt. f.<#> extract solution is believed to have herpesviral activity by itself.

Antiherpesviral activity was examined in combined use of Acv and an extract olution of the crude drug shown in Table 14.

As is apparent from the results in Table 14, antiherpesviral activity was displayed in the combined use of Acv and an extract solution of the crude drug showed significant synergism.

TEST EXAMPLE 11

Antiherpesviral activity of various crude drugs shown in Table 15 below in guinea pigs (p.o.) was determined as follows.

control group of guinea pigs was allowed to take water freely for 5 days. A 5 mg/mP extract solution of Cryt. f.<#> wad given to another group freely by mouth for 5 days. Still another group was allowed to take a 5 mg/mP solution of Areca catechu L. freely for 5 days. Other groups ware orally given 10 mP of a 20 mg/mP solution of each of the other crude drug extracts. Blood was taken from each animal, and an inactivated serum was prepared therefrom in the same manner as in Test Example 9. A plaque formation test was conducted using the resulting serum sample. The results obtained are shown in Table 15. It is obvious from the results that each crude drug extract exhibited antiherpesviral activity.

TEST EXAMPLE 12

Mouse was infected with Herpes simplex virus at the right midflank to examine the antiviral activity of the crude drug by a rate of herpes zoster formation and a rate of death.

In vivo antiviral activity of Geum japonicum Thunb. or Juglans mandshurica Maxim. in mice infected with herpes simplex virus was examined as follows.

The hair on the side abdomen of 6 to 7-week-old male BALB/c mice was removed by a chemical hair remover (produced by Shiseido Co., Ltd.). The hairless skin was scratched at random ten times with an intradermal injection needle 26G, and 10 NP of a herpes simplex virus I (Hayashida strain) (having been proliferated in Vero E6 cells) suspension (106PFU) was applied to the scarified area.

Immediately after the infection, 0.5 mP of the crude drug extract solution having a concentration of from 2 to 20 mg/mP(corresponding to 10 mg-drug/mouse) was orally given to the animal every 8 hours (3 times/day) for at least consecutive 10 days. A control group was orally given the equal volumes of water. The development of skin lesions and mortality were monitored three times a[illegible]y and any change observed on the infected skin was scored on the following b[illegible]s. The results obtained are shown in Table 16.

Score Standard: 0 ... No lesion 2 ... Vesicles in local region (bulla and erosion) 6 ... Mild zosteriform lesion 8 ... Moderate zosteriform lesion 10 .. Severe zosteriform lesion Death As is apparent from Table 16, the group having

Severe zosteriform lesion Death As is apparent from Table 16, the group having orally received the extract solution exhibited a reduced rate of death, an increased survival rate and a reduced rate of herpes zoster formation as compared with the control group, proving the antiherpesviral activity of the crude drug in vivo.

TABLE 16

(control)	6/6 (100%)	6/6 (100%)	8.20.4
Zeum japonicum Thunb.	3/4 (75%)	3/4 (75%)	10.21.8 *
Juglans mandshurica Maxim.	2/3 (67%)	2/3 (67%)	10.02.7

[Footnotes]
Note: *: significance level $p<0.05$

As described in the foregoing test examples, antiherpesviral activity of the crude drugs belonging to group A according to the present invention can be ascertained by the above-described in vitro screening test. Further, the serum prepared from the guinea pig having orally or intragastrointestinally received the extract of the crude drug of group B exhibited antiherpesviral activity. Furthermore, the crude drug extract having antiherpesviral activity obviously inhibited herpesvirus growth in mice (p.o.)(cf. Table 16). The results coincided with the results shown in Table 1 which was conducted in virto.

From all these test results, it was thus revealed that a crude drug of group which exhibits antiherpesviral activity in vitro also exhibits the same activity in vivo.

TEST EXAMPLE 13

Antipolioviral activity of Punica granatum L. (fruit peel) was determined by using the guinea pig serum prepared in the same manner as in Test Example 9. The results obtained are shown in Table 17. The results clearly demonstrate the antipolioviral activity of the serum of the Punica granatum group.

TABLE 17

	5 mg/mP		20 mg/mP
	9.0 x 106	9.5 x 106	7.25 x 106
b	9.0 x 106	8.5 x 106	4.75 X 106
c	1.48 x 107	8.25 x 106	6.25 x 105
d	1.05 x 107		8.25 x 106

d	1.05 x 107	-	8.25 x 106
e	-	-	5.75 X 106
Mean s	(1.080.274) x 107	(8.750.661) x 106 (p=0.265) *	(6.451.35) x 106 (p=0.016) *

n[Footnotes]
No[illegible]: *: t-Test value with respect to the normal serum.

Only the fruit peel of Punica grantum L. was used in this Example. The fruit peel of Punica grantum L. showed the same effect as the root bark thereof in the present invention. Punia grantum which can be obtained on the market is properly a++1999293106801mixture of the fruit peel and the root bark in this field.

TEST EXAMPLE 14

Antipolioviral activity of the crude drugs shown in Table 18 below in guinea pigs (p.o.) was determined in the same manner as in Test Example 11 as follows.

A group of guinea pigs was allowed to take water or a 5 mg/mP extract solution freely for 5 days. A serum sample was prepared in the same manner as in Test Example 9 and subjected to the plaque formation test. The results obtained are shown in Table 18. It is obvious from the results that each crude drug extract exhibited antipolioviral activity.

As described above, the antipolioviral activity of various crude drugs belonging to group C can be examined by in vitro screening (Test Example 2). The crude drugs of group C exhibited antipolioviral activity in the serum prepared from the guinea pig having orally or intragastrointestinally received the extract of the drug (Test Examples 13 and 14).

From these test results, it was thus revealed that a crude drug which exhibits antipolioviral activity in vitro also exhibits the same activity in vivo.

The dosage, administration route, etc. of the crude drugs included under group C are well known, and the known dose levels produce no or slight side effects. Accordingly, these crude drugs were proved effective to inhibit p[illegible]ovirus growth in oral administration or intragastrointestinal a[illegible]istration.

TEST EXAMPLE 15

The crude drugs exhibiting antiviral activity against both DNA viruses and

The crude drugs exhibiting antiviral activity against both DNA viruses and RNA viruses, i.e., the crude drugs of group G, are shown in Table 19 together with their data.

The crude drugs of group G were proved to exhibit antiviral activity against not only any species belonging to the family Herpesviridae but RNA viruses of different families (measles virus and poliovirus) and are therefore effective in the prevention and treatment of diseases caused by DNA viruses or RNA viruses.

The crude drugs in accordance with the present invention exhibit antiviral activity and inhibit Virus growth. Therefore, appropriate use of these crude drugs brings about improved effects in the prevention and treatment of virus infectious diseases.

While, in the foregoing test examples, the crude drugs of the present invention were used in the form of an aqueous extract or a methanol extract, extracts with other alcohols, e.g., ethyl alcohol, or a water/alcohol mixed solvent may also be used. A decoction or infusion prepared by decocting the crude drug with boiling water followed by filtration through a strainer, etc. can also be used. Further, a powder preparation prepared by grinding the crude drug is also usable.

While the invention has been described in detail and with reference to specific examples thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

ENGLISH-CLAIMS:

1. An antiviral agent containing at least one crude drug selected from the group consisting of (A1)the whole plant of Ainsliaea fragrans Champ., (A2)the rhizome of Alpinia officinarum Hance, (A3)the bark of Alyxia stellata Roem., A4)the bark of Andrographis paniculate Nees, (A5)the root of Andropogon izaniodes (L.) Urban, (A6)the rhizome of Anemarrhena asphodeloides Bunge, A7)the leaf of Arctostaphylos uva-ursi (L.) Sprengel, (A8)the seed of Areca atechu L., (A9)the leaf of Artemisia princeps Pamp., (A10)the whole plant of siasarum heterotropoides F. Maekawa var. mandshuricum F. Maekawa, (A11)the [illegible]me of Belamcanda chinensis (L.) DC., (A12)the rhizome of Brainia insignis [illegible].) J. Sm., (A13)the seed of Brucea javanica (L.) Merr., (A14)the root of upleurum fakatum L., (A15)the bark of Caesalpinia sappan L., (A16)the leaf of amellia japonica L., (A17)the bark of Cassia fistula L., (A18)the whole plant f Chamaesyce hyssopifolia, (A19)the bark and branch of Cinnamomum cassia Blume,

of Chamaesyce hyssopifolia, (A19)the bark and branch of Cinnamomum cassia Blume, (A20)the bark of Cinnamomum sintok Blumo, (A21)the rhizome of Cnidium officinale Makino, (A22)the rhizome of Coptis chinensis Franch., (A23)the leaf of Cordia spinescens, (A24)the fruit of Cornus officinalis Sieb. et Zucc., (A25)the tuber of Corydalis hurtscharinorii Besser forma yanhusuo Y.H. Chou et C.C. Hsu, (A26)the fruit of Curculigo orchioides Gaertn., (A27)the rhizome of Curcuma aeroginosa Roxb., (A28)the rhizome of Curcuma xanthorrhiza Roxb., (A29)the r ome of Cyrtomium fortunei J. Sm., (A30)the rhizome of Drynaria fortunei (zé) J. Smith, (A31)the rhizome of Dryopteris crassirhizoma Nakai, (A32)the fruit of Elaeocarpus grandiflorus Smith, (A33)the leaf of Elephantopus scaber L., (A34)the leaf of Epimedium koreanum Nakai, (A35)the leaf of Erythroxylum lucidum, (A35')the trunk of Erythroxylum citrifolium, (A36)the fruit of Evodia ruteacarpa Hook. f. et Thoms., (A37)the fruit of Foeniculum vulgare Mill., (A38)the fruit of Forsythia suspensa Vahl., (A39)the whole plant of Geranium thunbergii Sieb. et Zucc., (A40)the whole plant of Geum japonicum Thunb., (A41)the root and stolon of Glycyrrhiza uralensis Fisher (A42)the leaf of Hamelia axillaris Swartz, (A43)the branch and leaf of Jatropha curcas L., (A44)the bark of Juglans mandshurica Maxim., (A45)the root of Lithospermum erythrorhizon Sieb. et Zucc., (A46)the aerial part of Loranthus parasiticus (L.) Merr., (A47)the bark of Machilus thunbergii Sieb. et Zucc., (A48)the bark of Magnolia officinalis Rehd. et Wils., (A49)the rhizome of Matteuccia struthiopteris (L.) Todaro, (A50)the whole insect of Mylabris sidae Fabr., (A51)the root bark of Paeonia suffruticosa Andrews, (A52)the root of Panax ginseng C.A. Meyer, (A53)the bark of Parameria laevigata Moldenke, (A54)the leaf of Perilla frutescens Britton var. acuta Kudo, (A55)the bark of Phellodendron amurense Ruprecht, (A56)the aerial part of Physalis anqulata L., (A57)the rhizome of Plagiogyria matsumureana Makino, (A58)the root of Platycodon grandiflorum (Jacquin) A. DC., (A59)the root of Polygala tenuifolia Willd., (A60)the root and rhizome of Polygonum cuspidatum Sieb. et Zucc., (A61)the hoelen of Poria cocos Wolf, (A62)the spike of Prunella vulgaris L. subsp. asiatica Hara, (A63)the bark of Prunus jamasakura Siebold, (A64)the fruit of Prunus mume Sieb. et Zucc., (A65)the root bark and fruit peel of Punica granatum L., (A66)the bark of Quercus acutissima Carruthers, (A67)the leaf of Quercus salicina Blume, (A68)the fruit of Quisqualis indica L., (A69)the rhizome of Rheum palmatum L., (A70)the gall of Rhus javanica L., (A71)the root of Salvia miltiorrhiza Bunge, (A72)the leaf of Sarcandra glabra (Thunb.) Nakai, (A73)the flores of Schizonepeta tenuifoiia Briquet, (A74)the root of Scutellaria b alensis Georgi, (A75)the whole plant of Serjania mexicana, (A76)the flower b of Sophora japonica L., (A77)the root of Sophora subprostrata Chun et T. Chen, (A78)the stem of Spatholobus suberectus Dunn, (A79)the rhizome of Struthiopteris niponica (Kunze) Nakai, (A80)the seed of Strychnos nux-vomica L., (A81)the flower bud of Syzygium aromaticum (L.) Merr. et Perry, (A82)the

L., (A81)the flower bud of Syzygium aromaticum (L.) Merr. et Perry, (A82)the bark of Terminalia arjuna Wight et Arn., (A83)the fruit feel of Terminalia belerica Roxb., (A84)the fruit of Terminalia chebula Retzus, (A85)the leaf and branch of Uncaria gambir Roxb., (A86)the whole plant of Usnea misaminensis Vain., (A87)the branch and leaf of Waltheria indica L., (A88)the flower and leaf of Woodfordia floribunda Salisb., (A89)the rhizome of Woodwardia orientalis Sw., (A90)the fruit peel of zanthoxylum bungeanum Maxim., and (A91)the fruit of Z[illegible]phus jujuba Miller var. inermis Rehder.

2. An antiherpesviral agent containing at least one crude drug selected from the group consisting of (B1)the whole plant of Ainsliaea fragrans Champ., (B2)the rhizome of Alpinia officinarum Hance, (B3)the bark of Alyxia stellata Roem., (B4)the root of Andropogon zizaniodes (L.) Urban, (B5)the seed of Areca catechu L., (B6)the leaf of Artemisia princeps Pamp., (B7)the rhizome of Brainia insignis (Hook.) J. Sm., (B8)the seed of Brucea javanica (L.) Merr., (B9)the bark of Caesalpinia sappan L., (B10)the leaf of Camellia japonica L., (B11)the bark of Cassia fistula L., (B12)the whole plant of Chamaesyce hyssopifolia, (B13)the bark and branch of Cinnamomum cassia Blume, (B14)the bark of Cinnamomum tintok Blume, (B15)the rhizome of Coptis chinensis Franch., (B16)the leaf of Cordia spinescens, (B17)the rhizome of Cyrtomium fortunei J. Sm., (B18)the rhizome of Drynaria fortunei (Kunze) J. Smith, (B19)the rhizome of Dryopteris crassirhizoma Nakai, (B20)the fruit of Elaeocarpus grandiflorus Smith, (B21)the leaf of Epimedium koreanum Nakai, (B22)the leaf of Erythroxylum lucidum, (B22')the trunk of Erythroxylum citrifolium, (B23)the fruit of Foeniculum vulgare Mill., (B24)the whole plant of Geranium thunbergii Sieb. et Zucc., (B25)the whole plant of Geum japonicum Thunb., (B26)the leaf of Hamelia axillaris Swartz, (B27)the branch and leaf of Jatropha curcas L., (B28)the bark of Juglans mandshurica Maxim., (B29)the bark of Machilus thunbergii Sieb. et Zucc., (B30)the root bark of Paeonia suffruticosa Andrews, (B31)the leaf of Perilla frutescens Britton var. acuta Kudo, (B32)the bark of Phellodendron amurense Ruprecht, (B33)the rhizome of Plagiogyria matsumureana Makino, (B34)the root of Polygala tenuifolia Willd., (B35)the root and rhizome of Polygonum cuspidatum Sieb. et Zucc., (B36)the spike of Prunella vulgaris L. subsp. asiatica Hara, (B37)the root bark and fruit peel of Punica granatum L., (B38)the bark of Quercus acutissima Carruthers, (B39)the rhizome of Rheum palmatum L., (B40)the gall of Rhus javanica L., (B41)the root of Salvia miltiorrhiza Bunge, (B42)the leaf of Sarcandra glabra (Thunb.) Nakai, (B43)the flores of [illegible]onepeta tenuifolia Briquet, (B44)the whole plant of Serjania mexicana, [illegible], (B45)the stem of Spatholobus suberectus Dunn, (B46)the bark of Terminalia arjuna Wight et Arn., (B47)the fruit peel of Terminalia belerica Roxb., (B48)the fruit of Terminalia chebula Retzus, (B49)the branch and leaf of Waltheria indica L., (B50)the flower and leaf of Woodfordia floribunda Salisb., (B51)the rhizome of

B50)the flower and leaf of Woodfordia floribunda Salisb., (B51)the rhizome of oodwardia orientalis Sw., and (B52)the fruit peel of Zanthoxylum bungeanum laxim.

3. An antipolioviral agent containing at least one crude drug selected from he group consisting of (C1)the rhizome of Alpinia officinarum Hance, (C2)the ark of Andrographis paniculate Nees, (C3)the root of Andropogon zizaniodes (L.) , (C4)the rhizome of Anemarrhena asphodeloides Bunge, (C5)the leaf of staphylos uva-ursi (L.) Sprengel, (C6)the seed of Areca catechu L., (C7)the eaf of Artemisia princeps Pamp., (C8)the whole plant of Asiasarum etertropoides F. Maekawa var. mandshuricum F. Maekawa, (C9)the rhizome of elamcanda chinensis (L.) DC., (C10)the rhizome of Brainia insignis (Hook.) J. m., (C11)the seed of Brucea javanica (L.) Merr, (C12)the bark of Caesalpinia appan L., (C13)the bark of Cassia fistula L., (C14)the bark of Cinnamomum intok Blume, (C15)the rhizome of Coptis chinensis Franch., (C16)the fruit of ornus officinalis Sieb. et Zucc., (C17)the tuber of Corydalis hurtscharinorii esser forma yanhusuo Y.H. Chou et C.C. Hsu, (C18)the fruit of Curculigo rchioides Gaertn., (C19)the rhizome of Curcuma aeroginosa Roxb., (C20)the hizome of Curcuma xanthorrhiza Roxb., (C21)the rhizome of Cyrtomium fortunei J. m., (C22)the rhizome of Dryopteris crassirhizoma Nakai, (C23)the fruit of laeocarpus grandiflorus Smith, (C24)the leaf of Elephantopus scaber L., C25)the fruit of Evodia rutaecarpa Hook. f. et Thoms., (C26)the fruit of oeniculum vulgare Mill., (C27)the fruit of Forsythia suspensa Vahl., (C28)the hole plant of Geranium thunbergii Sieb. et Zucc., (C29)the whole plant of Geum aponicum Thunb., (C30)the bark of Juglans mandshurica Maxim., (C31)the root of ithospermum erythrorhizon Sieb. et Zucc., (C32)the aerial part of Loranthus arasiticus (L.) Merr., (C33)the bark of Machilus thunbergii Sieb. et Zucc., C34)the rhizome of Matteuccia struthiopteris (L.) Todaro, (C35)the whole insect f Mylabris sidae Fabr., (C36)the root bark of Paeonia suffruticosa Andrews, C37)the bark of Parameria laevigata Moldenke, (C38)the bark of Phellodendron murense Ruprecht, (C39)the aerial part of Physalis angulata L., (C40)the hizome of Plagiogyria matsumureana Makino, (C41)the root of Polygala tenuifolia illd., (C42)the root and rhizome of Polygonum cuspidatum Sieb. et Zucc., C43)the bark of Prunus jamasakura Siebold, (C44)the fruit of Prunus mume Sieb. Zucc., (C45)the root bark and fruit peel of Punica granatum L., (C46)the bark f Quercus acutissima Carruthers, (C47)the leaf of Quercus salicina Blume, C48)the fruit of Quisqualis indica L., (C49)the rhizome of Rheum palmatum L., the gall of Rhus javanica L., (C51)the root of Scutellaria baicalensis gi, (C52)the flower bud of Sophora japonica L., (C53)the root of Sophora bprostrata Chun et T. Chen, (C54)the stem of Spatholobus suberectus Dunn, C55)the rhizome of Struthiopteris niponica (Kunze) Nakai, (C56)the seed of rychnos nux-vomica L., (C57)the flower bud of Syzygium aromaticum (L.) Merr.

Strychnos nux-vomica L., (C57)the flower bud of Syzygium aromaticum (L.) Merr. et Perry, (C58)the bark of Terminalia arjuna Wight et Arn., (C59)the fruit peel of Terminalia belerica Roxb., (C60)the fruit of Terminalia chebula Retzus, (C61)the leaf and branch of Uncaria gambir Roxb., (C62)the whole plant of Usnea nisaminensis Vain., (C63)the flower and leaf of Woodfordia floribunda Salisb, (C64)the rhizome of woodwardia orientalis Sw., and (C65)the fruit peel of Zanthoxylum bungeanum Maxim.

. An anti-measles virus agent containing at least one crude drug selected om the group consisting of (D1)the seed of Areca catechu L., (D2)the leaf of rtemisia princeps Pamp., (D3)the rhizome of Belamcanda chinensis (L.) DC., D4)the rhizome of Brainia insignis (Hook.) J. Sm., (D5)the seed of Brucea avanica (L.) Merr, (D6)the bark of Caesalpinia sappan L., (D7)the bark of assia fistula L., (D8)the bark of Cinnamomum sintok Blume, (D9)the rhizome of nidium officinale Makino, (D10)the rhizome of Coptis chinensis Franch., D11)the rhizome of Cyrtomium fortunei J. Sm., (D12)the fruit of Elaeocarpus randiflorus Smith, (D13)the leaf of Elephantopus scaber L., (D14)the fruit of oeniculum vulgare Mill,, (D15)the fruit of Forsythia suspensa Vahl., (D16)the hole plant of Geranium thunbergii Sieb. et Zucc., (D17)the root and stolon of lycyrrhiza uralensis Fisher, (D18)the bark of Juglans mandshurica Maxim., D19)the bark of Magnolia officinalis Rehd. et Wils., (D20)the whole insect of ylabris sidae Fabr., (D21)the root bark of Paeonia suffruticosa Andrews, D22)the bark of Phellodendron amurense Ruprecht, (D23)the rhizome of lagiogyria matsumureana Makino, (D24)the root of Platycodon grandiflorum Jacquin) A. DC., (D25)the root of Polygala tenuifolia Willd., (D26)the root ark and fruit peel of Punica granatum L., (D27)the bark of Quercus acutissima arruthers, (D28)the rhizome of Rheum palmatum L., (D29)the gall of Rhus ivanica L., (D30)the root of Scutellaria baicalensis Georgi, (D31)the stem of patholobus suberectus Dunn, (D32)the flower bud of Sysygium aromaticum (L.) err. et Perry, (D33)the bark of Terminalia arjuna Wight et Arn., (D34)the fruit eel of Terminalia belerica Roxb., (D35)the fruit of Terminalia chebula Retzus,)36)the flower and leaf of Woodfordia floribunda Salisb., (D37)the rhizome of oodwardia orientalis Sw., and (D38)the fruit of Zizyphus jujuba Miller var. ermis Rehder.

5. An anti-varicella-zoster virus agent containing at least one crude drug lected from the group consisting of (E1)the rhizome of Alpinia officinarum e, (E2)the seed of Areca catechu L., (E3)the leaf of Artemisia princeps ., (E4)the root of Bupleurum fakatum L., (E5)the bark of Cassia ;0521093106801stula L., (E6)the rhizome of Coptis chinensis Franch., (E7)the izome of Cyrtomium fortunei J. Sm,, (E8)the rhizome of Drynaria fortunei unze) J. Smith, (E9)the whole plant of Geranium thunbergii Sieb, et Zucc.,

(Kunze) J. Smith, (E9)the whole plant of Geranium thunbergii Sieb, et Zucc., (E10)the root and stolon of Glycyrrhiza uralensis Fisher, (E11)the bark of Juglans mandshurica Maxim., (E12)the root bark of Paeonia suffruticosa Andrews, (E13)the root of Panax ginseng C.A. Meyer, (E14)the bark of Phellodendron amurense Ruprecht, (E15)the rhizome of Plagiogyria matsumureana Makino, (E16)the root of Polygala tenuifolia Willd., (E17)the root and rhizome of Polygonum cuspidatum Sieb. et Zucc., (E18)the hoelen of Poria cocos Wolf, (E19)the root bark and fruit peel of Punica granatum L., (E20)the bark of Quercus acutissim ruthers, (E21)the rhizome of Rheum palmatum L., (E22)the gall of Rhus javanica L., (E23)the flower bud of Syzygium aromaticum (L.) Merr. et Perry, (E24)the bark of Terminalia arjuna Wight et Arn., (E25)the fruit of Terminalia chebula Retzus, (E26)the rhizome of Woodwardia orientalis Sw., and (E27)the rhizome of Dryopteris crassirhizoma Nakai.

6. An anti-CMV agent containing at least one crude drug selected from the group consisting of (F1)the seed of Areca catechu L., (F2)the leaf of Artemisia princeps Pamp., (F3)the bark of Cassia fistula L., (F4)the rhizome of Coptis chinensis Franch., (F5)the rhizome of Cyrtomium fortunei J. Sm., (F6)the rhizome of Drynaria fortunei (Kunze) J. Smith, (F7)the whole plant of Geranium thunbergii Sieb. et Zucc., (F8)the whole plant of Geum japonicum Thunb., (F9)the bark of Juglans mandshurica Maxim., (F10)the bark of Machilus thunbergii Sieb. et Zucc., (F11)the root bark of Paeonia suffruticosa Andrews, (F12)the bark of Phellodendron amurense Ruprecht, (F13)the rhizome of Plagiogyria matsumureana Makino, (F14)the root of Polygala tenuifolia Willd., (F15)the root and rhizome of Polygonum cuspidatum Sieb. et Zucc., (F16)the root bark and fruit peel of Punica granatum L., (F17)the bark of Quercus acutissima Carruthers, (F18)the rhizome of Rheum palmatum L., (F19)the gall of Rhus javanica L., (F20)the flower bud of Syzygium aromaticum (L.) Merr. et Perry, (F21)the bark of Terminalia arjuna Wight et Arn., (F22)the fruit of Terminalia chebula Retzus, and (F23)the rhizome of Woodwardia orientalis Sw.

7. An anti-DNA virus and anti-RNA virus agent containing at least one crude drug selected from the group consisting of (G1)the seed of Areca catechu L., (G2)the leaf of Artemisia princeps Pamp., (G3)the rhizome of Brainia insignis (Hook.) J. Sm., (G4)the seed of Brucea javanica (L.) Merr., (G5)the bark of Caesalpinia sappan L., (G6)the bark of Cassia fistula L., (G7)the bark of Cinnamomum sintok Blume, (G8)the rhizome of Coptis chinensis Franch., (G9)the rhizome of Cyrtomium fortunei J. Sm., (G10)the fruit of Elaeocarpus grandiflorus , (G11)the fruit of Foeniculum vulgare Mill., (G12)the whole plant of Geranium thunbergii Sieb. et Zucc., (G13)the bark of Juglans mandshurica Maxim., (G14)the root bark of Paeonia suffruticosa Andrews, (G15)the bark of Phellodendron amurense Ruprecht, (G16)the rhizome of Plagiogyria matsumureana

Phellodendron amurense Ruprecht, (G16)the rhizome of Plagiogyria matsumureana Makino, (G17)the root of Polygala tenuifolia Willd., (G18)the root bark and fruit peel of Punica granatum L., (G19)the bark of Quercus acutissima Carruthers, (G20)the rhizome of Rheum palmatum L., (G21)the gall of Rhus javanica L., (G22)the stem of Spatholobus suberectus Dunn, (G23)the flower bud of Syzygium aromaticum (L.) Merr. et Perry, (G24)the bark of Terminalia arjuna Wight et Arn., (G25)the fruit peel of Terminalia belerica Roxb., (G26)the fruit of Terminalia chebula Retzus, (G27)the flower and leaf of Woodfordia floribunda Salisb., and (G28)the rhizome of Woodwardia orientalis Sw.

8. An antiherpesviral agent containing at least one crude drug selected from the group B of the claim 2 and the other antiviral agent.

LOAD-DATE: February 24, 2000

LEVEL 1 - 29 OF 50 ABSTRACTS

PATENT ABSTRACTS OF JAPAN

05194246

August 3, 1993

PRODUCTION OF HERB MEDICINAL HARD CAPSULE

VENTOR: UCHIDA TOSHIHIRO; KONISHI SHINICHIRO; KIMURA TAKAYOSHI

PL-NO: 04255406 (JP 92255406)

LED: September 1, 1992

ORITY: September 5, 1991, 40325274, Japan (JP)

SIGNEE: TSUMURA & CO

T-CL: A61K35/78, (Section A, Class 61, Sub-class K, Group 35, Sub-group 78); 61K9/48, (Section A, Class 61, Sub-class K, Group 9, Sub-group 48)

BST:
PURPOSE: To obtain the subject herb medicinal hard capsule, having a high tract content and a short elution time of the contents and excellent in ution properties.

CONSTITUTION: The objective herb medicinal hard capsule is obtained by adding gnesium stearate in an amount of 0.01-5wt. %, preferably 0.5-3.0wt. % based on extract powder to particles prepared by compacting dry extract powder of a b medicine. This herb medicinal hard capsule is good in eluting properties of ickly eluting the contents. Furthermore, the herb medicine is not especially ited; however, e.g. ORENGEDOKUTO (a decoction containing Coptidis Rhizoma, llondedri Cortex, Scutellariae Radix, etc.), NINJINTO (a decoction aining Ginseng Radix, Glycyrrhizae Radix, etc.), SHOSAIKOTO (a decoction itaining Bupleuri Radix, Pinelliae Tuber, Scutellariae Radix, etc.), BUSHISAISHINTO (a decoction containing Ephedrae Herba, Asiasari Radix, niti Sinensis Tuber, etc.), ANCHUSAN (powder containing Cinnamomi Cortex, ydalis Tuber, Ostreae Testa, Foeniculi Fructus, etc.), KEISHIBURYOGAN (a l containing Cinnamomi Cortex, Hoelen, Moutan Cortex, etc.), KKOKANINJINTO (a decoction containing Anemarrhenae Rhizoma, Gypsum,

KOKANINJINTO (a decoction containing Anemarrhenae Rhizoma, Gypsum, yrrhizae Radix, Ginseng Radix, etc.), SHAKUYAKUKANZOTO (a decoction aining Paeoniae Radix, Glycyrrhizae Radix, etc.) and SANOSHASHINTO (a ction containing Rhei Rhizoma, Scutellariae Radix, Coptidis Rhizoma, etc.) preferred.

LEVEL 1 - 1 OF 50 ABSTRACTS

PATENT ABSTRACTS OF JAPAN

02264663

October 29, 1990

AROMATIC DEODORANT

INVENTOR: NIWA AKIRA

APPL-NO: 01083949 (JP 89083949)

FILED: April 4, 1989

ASSIGNEE: NIWA AKIRA

INT-CL: A61L9/01, (Section A, Class 61, Sub-class L, Group 9, Sub-group 01)

ABST:

PURPOSE: To suppress the excessive secretion of secretion and to impart comfortable aromas by incorporating the crude drug extracts of chrysanthemum flowers, Japanese honeysuckle, broomrape, scutellaria, gallnut, Japanese indigo plant, areca nut, cassia seed, atractylodes, licorice and refined Berneo camphor into the deodorant.

CONSTITUTION: The arom. deodorant is formed by compounding 30 pars chrysanthemum flowers, 30 parts Japanese honeysuckle, 30 parts broomrape, 20 parts scutellaria, 30 parts gallnut, 20 parts Japanese indigo plant, 20 parts areca nut, 30 parts cassia seed, 30 parts atractyloldes, 15 parts licorice, and 3 parts refined Borneo camphor of the respective crude drug extracts. The chrysanthemum flowers has antipyretic, antiinflammatory, detoxicant and divergent effects; the Japanese honeysuckle has urination and detoxicant effects; the broomrape acts as an invigorating drug; the scutellaria has i[illegible]ammatory, antipyroretic, detoxicant, and hemostatic effects; the gallnut has h[illegible]ostatic, astringent and fermentation suppressing effects; the Japanese indigo plant has antipyretic and detoxicant effects; the areca nut has astringent, peptic property, water blister resistance, insecticidal destruction effects; the cassia seed has blood pressure lowering and urination effects; the atractylodes has urination and perspiration effects; the licorice has detoxicant and antiinflammatory effect; the refined Borneo camphor has incense effects, etc.

LEVEL 1 - 47 OF 50 ABSTRACTS

PATENT ABSTRACTS OF JAPAN

09286736

November 4, 1997

LIQUID DRUG FOR INTERNAL USE

VENTOR: OKUDAIRA ICHIRO; TSUNODA KENJI

PL-NO: 08100790 (JP 96100790)

ED: April 23, 1996

SIGNEE: TAISHO PHARMACEUT CO LTD

T-CL: A61K35/78, (Section A, Class 61, Sub-class K, Group 35, Sub-group 78); 61K35/78, (Section A, Class 61, Sub-class K, Group 35, Sub-group 78); 61K35/78, (Section A, Class 61, Sub-class K, Group 35, Sub-group 78); A61K9/08, ection A, Class 61, Sub-class K, Group 9, Sub-group 08); A61K47/26, (Section Class 61, Sub-class K, Group 47, Sub-group 26)

BST:

PROBLEM TO BE SOLVED: To obtain Saiko-keishito (a Chinese herbal drug) fective for the mitigation of various symptoms of cold in the form of a liquid ug for internal use having good taste and flavor by compounding a specific eetener of natural origin to an extract of Saiko-Keishito known as an cellent herbal drug.

SOLUTION: An extract of Saiko-keishito is compounded with stevia. iko-keishito is a Chinese herbal drug preparation composed of Saiko (root of leurum falcatum), Hange (rhizome of Pinellia ternata), Keishi (twig of namomum cassia), Shakuyaku (root of Paeonia albiflora), Ougon (root of tellaria baicalensis), Ninjin (root of Panax ginseng), Taisou (fruit of yphus vulgaris), Kanzou (root of Glycyrrhiza glabra) and Kanshoukyou izome of Zingiber officinale). Stevia is a sweetener composed mainly of a eetener component existing in the leaf of Stevia Rebaudiana Bertoni which is a ennial plant of the family Compositae. The main components of the sweetener stevioside, rebaudioside A, dulcoside A, rebaudioside E, steviol, etc., and ecially a sweetener composed of 100% rebaudioside A is most preferable. The

LEVEL 1 - 34 OF 50 ABSTRACTS

PATENT ABSTRACTS OF JAPAN

63239228

October 5, 1988

IMMUNOACTIVATING AGENT

VENTOR: FUJIMAKI MICHIO; IKEMATSU MASAJIRO; HANEDA MASAO; FUKUE HIDENAO; KUTAKE KATSUHIRO

PL-NO: 62073705 (JP 87073705)

LED: March 27, 1987

SIGNEE: TSUMURA & CO

T-CL: A61K35/78, (Section A, Class 61, Sub-class K, Group 35, Sub-group 78); 1K35/78, (Section A, Class 61, Sub-class K, Group 35, Sub-group 78)

BST:
PURPOSE: To obtain an immunoactivating agent for person infected by virus of quired immune deficiency syndrome, containing the Chinese medicine SHOSAIKOTO lend of Bupleuri radix, etc. with other ingredients) and having extremely w toxicity and high safeness.

CONSTITUTION: A diluting agent, auxiliary agent, etc., used in a conventional rmulation is added to a dried extract powder of SHOSAIKOTO (a Chinese medicine oduced from blend of Bupleuri radix with the following ingredients) produced cording to blend ratio of crude drugs described in old books such as SHOKANRON d KINKIYORYAKU, and formulated by a conventional method to provide the aimed ent. The agent can be prepared in a form such as inhalant, granule, tablet, sule agent, etc., and administered in dose of 1W10g / day / head three times n amount of dried extract powder. A blend ratio of each crude medicine of e above-mentioned BHOSAIKOTO is illus trated by 4W7pts.wt. Bupleuri Radix, ts.wt. Scutellariae Radix, 2pts.wt. Glycyrrhizae Radix, 2W3pts.wt. Ginseng adix, 1pt.wt. Zingiberis, 2W3pts.wt. Zizyphi Fructus and 4W5pts.wt. nelliae Tuber.